From the Department of Clinical Science and Education,
Södersjukhuset
Karolinska Institutet, Stockholm, Sweden

SENTINEL LYMPH NODE BIOPSY IN BREAST CANCER-ASPECTS ON INDICATIONS AND LIMITATIONS

Linda Holmstrand Zetterlund

Karolinska Institutet

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Sentinel lymph node biopsy in breast cancer – Aspects on indications and limitations

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Linda Holmstrand Zetterlund

Huvudhandledare:
MD PhD Fuat Celebioglu
Karolinska Institutet
Institutionen för klinisk forskning och utbildning

Bihandledare:
Professor Rimma Axelsson
Karolinska Institutet
Institutionen för klinisk vetenskap, intervention och teknik

Docent Jana de Boniface
Karolinska Institutet
Institutionen för molekylär medicin och kirurgi

Professor Jan Frisell
Karolinska Institutet
Institutionen för molekylär medicin och kirurgi

Fakultetsopponent:
Ellen Schlichting, MD, PhD
Oslo Universitet
Institutt for klinisk medisin, Medicinsk fakultet

Betygsnämnd:
Docent Signe Borgquist
Lunds Universitet
Institutionen för kliniska vetenskaper, Avdelningen för onkologi och patologi

Docent Jan Zedenius
Karolinska Institutet
Institutionen för molekylär medicin och kirurgi

Johan Lindholm
Karolinska Institutet
Institutionen för onkologi och patologi

Stockholm 2017

Till Fredrik, Klara och Hugo

ABSTRACT

Axillary lymph node status is the most important prognostic factor in breast cancer. Sentinel lymph node biopsy (SLNB) was introduced in the late 1990s and has replaced axillary lymph node dissection (ALND) as the gold standard axillary nodal staging procedure in early breast cancer due to higher accuracy and less morbidity compared with ALND. The overall aim of this thesis was to investigate SLNB and its current role in breast cancer today with a focus on current controversies and its limitations in different clinical settings.

The first paper (I) is a national registry study investigating the incidence of positive sentinel lymph nodes (SLNs) in women with a postoperative diagnosis of pure DCIS (ductal carcinoma in situ). We also investigated whether additional tumor sectioning could reveal occult tumor invasion among the patients with tumor deposits in their SLNs. SLNB was performed in 753 patients of whom 11 had tumor deposits in their SLNs. Two patients had macro- and three micrometastasis (N1). Six patients had isolated tumor cells (N0(i+)), resulting in a SLN positive rate of 0.7% (5/753). We did not find any risk factors for SLN metastasis. Occult invasion was found to the same extent among patients with SLN metastasis 9% (1/11) as in the matched control group of 10% (2/21).

The aim of the second paper (II) was to evaluate lymph drainage patterns to the axillary lymph nodes with hybrid SPECT/CT imaging before, compared with six weeks after a diagnostic breast excision of an unsuspicious breast tumor. SPECT (single photon emission computed tomography) integrates nuclear medicine imaging with CT (computed tomography) which results in functional images with precise anatomical localization of radioactive SLNs. The contralateral breast served as a control. The SLN detection rate was 91.9% (34/37) on operated sides postoperatively compared with 93.7% (104/111) on non-operated sides, p=0.0771. Partial or total concordance regarding number and localization of radioactive lymph nodes was not significantly lower on operated at 85.7% (30/35) compared with 88.9% (32/36) on non-operated sides, P=0.735.

In the third (III) and fourth (IV) papers SLNB in the neoadjuvant settting was evaluated in a Swedish prospective multicenter trial recruiting women with biopsy-verified breast cancer planned for neoadjuvant systemic therapy (NAST). In paper III clinically node-negative (cN0) patients were enrolled and SLNB performed prior to commencement of NAST. A completion ALND was performed in all patients in both trial arms. The identification rate (IR) was 100% (224/224). The proportion of patients with a negative SLNB but still positive lymph nodes in the axilla after NAST was 7.4% (9/121, 95%, CI: 4.0-13.5). In paper IV, SLNB was attempted after NAST in 195 patients with biopsy-proven node-positive breast cancer at stage T1-4d. The overall IR was 77.9% (152/195) and the overall FNR 14.1 % (13/92). The FNR decreased to 4.0% when two or more SLNs were retrieved.

Conclusions: Positive SLNs are rare in pure DCIS. SLNB should only be performed if mastectomy is planned or in case of high risk of invasive disease if breast-conserving surgery is planned. SLNB after prior diagnostic surgery seems accurate with minor impact on lymph drainage patterns. SLNB in cN0 patients before NAST is highly reliable. SLNB after NAST in clinically node-positive patients with T1-4d stage breast cancer is feasible but associated with lower IR and higher FNR than in clinically node-negative patients. Only if two or more SLNs are retrieved can the omission of ALND be considered.

LIST OF PUBLICATIONS

I. **Zetterlund L, Stemme S, Arnrup H, de Boniface J.**
Incidence of and risk factors for sentinel lymph node metastasis in patients with a postoperative diagnosis of ductal carcinoma in situ
Br J Surg. 2014; 101: 488-494

II. **Zetterlund L, Gabrielson S, Axelsson R, De Boniface J, Frisell J, Olsson A, Celebioglu F.**
Impact of previous surgery on sentinel lymph node mapping: hybrid SPECT/CT before and after a unilateral diagnostic breast excision
The Breast 30 2016; 30: 32-38

III. **Zetterlund L, Celebioglu F, Axelsson R, de Boniface J, Frisell J.**

Swedish prospective multicenter trial on the accuracy and clinical relevance of sentinel lymph node biopsy before neoadjuvant systemic therapy in breast cancer
Breast Cancer Res Treat. Published on line: 17 Feb 2017

IV. **Zetterlund LH, Frisell J, Zouzos A, Axelsson R, Hatschek T, de Boniface J, Celebioglu F.**
Swedish prospective multicenter trial evaluating sentinel lymph node biopsy after neoadjuvant systemic therapy in clinically node-positive breast cancer
Breast Cancer Res Treat. Published on line: 21 Feb 2017

LIST OF ABBREVIATIONS

AJCC	American Joint Committee on Cancer
ALH	Atypical lobular hyperplasia
ALND	Axillary lymph node dissection
AUS	Axillary ultrasound
BCS	Breast-conserving surgery
CT	Computed tomography
DCIS	Ductal carcinoma in situ
DFS	Disease-free survival
ER	Estrogen receptor
FNAC	Fine needle aspiration cytology
FNR	False negative rate
HER2	Human epidermal growth factor receptor 2
HRT	Hormone replacement therapy
IBC	Inflammatory breast cancer
IHC	Immunohistochemical
IR	Identification rate
ITC	Isolated tumor cells
HER2	Human epidermal growth factor receptor 2
LABC	Locally advanced breast cancer
LCIS	Lobular carcinoma in situ
LN	Lobular neoplasia
MRI	Magnetic resonance imaging
NAC	Neoadjuvant chemotherapy
NAST	Neoadjuvant systemic therapy
NPV	Negative predictive value
PCR	Pathological Complete Response
NST	No special type
PET	Positron emission tomography

PLCIS	Pleomorphic lobular carcinoma in situ
PPV	Positive predictive value
PgR	Progesteron receptor
RCB	Residual cancer burden
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy
SPECT	Single photon emission computed tomography
SPIO	Supraparamagnetic iron oxide
TNM	Tumor, node and metastasis

CONTENTS

1 INTRODUCTION

I was introduced to the field of research by Fuat Celebioglu, the head of the Breast Surgical Unit at Södersjukhuset in 2009, less than a year after having started my subspecialisation on breast surgery. At that time, my children were five and two years old respectively and if had known then, what I know now, namely how much precious time, effort and sacrifices it takes to become a doctor of philosophy, I am not sure that I would have accepted the invitation. Even though there has been a lot of hard work along the way, I am glad that I decided to embark opon this long journey.

In September 2009, I myself together with Fuat Celebioglu, professor Jan Frisell, Karolinska University Hospital Solna and professor Leif Bergkvist, Västerås Hospital met for the first time to discuss the study design for a new national research trial. They are all senior breast surgeons with a scientific special interest in SLNB in breast cancer. Fuat Celebioglu was to become my main supervisor and professor Jan Frisell my first co-supervisor.

This was the start of the Swedish prospective multicenter trial with the aim of evaluating the feasibility and timing of SLNB within the neoadjuvant setting in breast cancer, as requested by the Swedish Society for Breast Surgery. The study opened for accrual in October 2010 and did not close until the end of December 2015 after having reached its accrual goal. The results of this study formed the basis for papers III and IV.

I became a registered doctoral student in October 2011 and the year before, I was introduced to professor Rimma Axelsson, Department of Radiology, Karolinska University Hospital Huddinge, who became my second co-supervisor. She had been collaborating with Fuat Celebioglu in research projects investigating lymph drainage patterns evaluated with scintigraphic imaging after axillary surgery. Professor Axelsson and Fuat Celebioglu had plans for a new study investigating lymph drainage alterations after a surgical breast biopsy evaluated with hybrid SPECT/CT imaging. The results of this project formed the basis for paper II.

Last but certainly not least, professor Jan Frisell introduced me to his breast surgical colleague, associate professor Jana de Boniface, my third co-supervisor. She had started to collect data from the Swedish National Breast Cancer Registry to be used for a retrospective study with the principal aim of investigating the incidence of SLN metastasis in women with a postoperative diagnosis of pure DCIS. DCIS is a precancerous entity of breast cancer theoretically not able to metastasize. The results of this project formed the basis for paper I.

2 BACKGROUND

2.1 ANATOMY OF THE BREAST

The breast starts to develop during fetal week six as a thickening on the chest called the mammary ridge or milk line. Several epithelial buddings develop along the milk line but only two remain and by the time the baby is born two nipples and the beginning of a duct system with 15-20 main ducts have formed. At puberty the female breast enlarges slowly under influence of estrogens from the ovaries and the duct system continues to branch and grow. In addition, fat and fibrous tissue will grow in between the mammary ducts. Fibrous septa within the breast, continuous with the underlying deep fascia, develop and function as an "inner bra" giving support to the breast. These septa or suspensory ligaments are called Cooper's ligaments. The breast is richly vascularized from several arteries. The majority of the blood is delivered by perforants from the internal mammary artery. The venous blood follows the arteries and drains into the internal thoracic and axillary veins. There are several nerves that run within the axillary region at risk of getting injured during axillary surgery. The thoracicus longus nerve innervates the anterior serratus muscle and an injured nerve will result in a winged scapula. The thoracodorsal nerve innervates the latissmus dorsi muscle which is involved in adduction and extension of the arm. There are also intercostobrachial sensory nerves innervating the skin in the lateral aspect of the axilla and the medial part of the upper arm.

It is not until the completion of the first pregnancy that the breast matures fully with further branching and growing of the ducts into lobes and smaller lobules and finally at their ends, the secretory alveoli where the milk production takes place. The breast parenchyma consists of 15-20 lobes, each draining into a major lactiferous duct which dilates to as sinus behind the areola. When lactation ceases the glandular tissue regresses but not as far as to the pattern before the first pregnancy. This process continues after the menopause with further loss of alveoli and reduction of the duct system (involution) and eventually the glandular tissue is mostly replaced by fat [1].

2.1.1 The lymphatic system

The primary function of the lymphatic system is to drain interstitial fluid from small blind-ending lymphatic capillaries consisting of a single layer of endothelial cells. An osmotic pressure gradient and smooth muscle contractions generate lymph flow. Lymphatic capillaries drain into precollectors containing valves helping to direct the lymph which in turn drains into afferent lymphatic vessels on the cortex of the lymph nodes. Throughout the lymphatic system, lymph nodes are distributed, functioning as filters. The lymph leaves the node from the hilum in efferent lymphatic vessels that drain into larger collecting vessels which in turn eventually reach the thoracic duct or the right lymph duct before draining into the venous circulation via a jugular anastomosis [2].

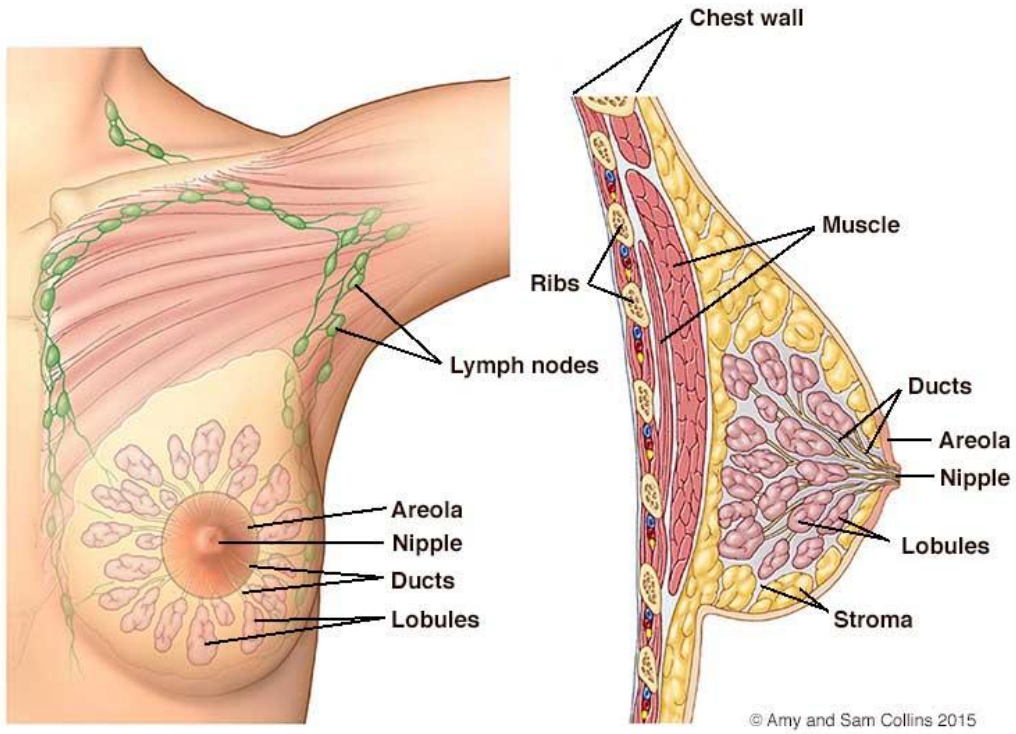

Figure 1 *Anatomy of the breast. Reproduced with permission from Komen Greater NYC.*

Since the introduction of the sentinel lymph node biopsy (SLNB) technique, there has been an increased interest in the lymphatic system and lymph drainage from the breast. The lymph vessels in the breast are arranged in a superficial and a deep lymphatic system. The superficial lymphatics in the skin and nipple communicate with a subareolar lymphatic plexus. According to studies by Sappey in the 1830s using mercury injections in the lymphatic system [3], lymph from deeper parts within the breast tissue drain centripetally into the subareolar plexus on its way to the axilla. Later, studies by Turner-Warwick in 1959 using colloidal gold, show that the deep lymphatics in the breast parenchyma arising from the lobules, to some extent also drain directly to the axilla without first passing through the subareolar plexus, although connections between the deep and superficial collecting lymph vessels exist [4]. In addition, about 20% of the lymph in the deep lymph vessels follow branches of the internal thoracic artery and drain into the parasternal internal mammary chain nodes [5]. The ipsilateral axillary lymph nodes receive about 75 % of the lymph from the breast and are the most common site for metastases from a breast cancer [1]. Other less common drainage routes are to the ipsilateral internal mammary, supraclavicular and posterior intercostal nodes and less frequently to the contralateral parasternal or axillary nodes [6].

The axillary lymph nodes are usually grouped into level I (below and lateral to the pectoralis minor muscle), level II (behind the pectoralis minor muscle) and level III (medial to the pectoralis minor and below the clavicle).

2.2 EPIDEMIOLOGY AND RISK FACTORS FOR BREAST CANCER

Breast cancer is the most common cancer in women in the western world and accounts for approximately 30% of female malignancies. The incidence is high in Northern Europe, North America and Australia and low in Asia and Africa but has increased globally over the last decades, especially in high income countries [7]. The increase has been ascribed to altered reproductive patterns (such as later age at first birth and less use of breast-feeding), increased use of menopausal hormone replacement therapy (HRT) and mammography screening programmes. During the latest 20 years, the annual increase in incidence in Sweden has been 1.6%. The largest increase is seen in women aged 60-69 years [8]. The median age at diagnosis is 65 years. Time trends for incidence and mortality for female breast cancer is shown in Figure 2 below.

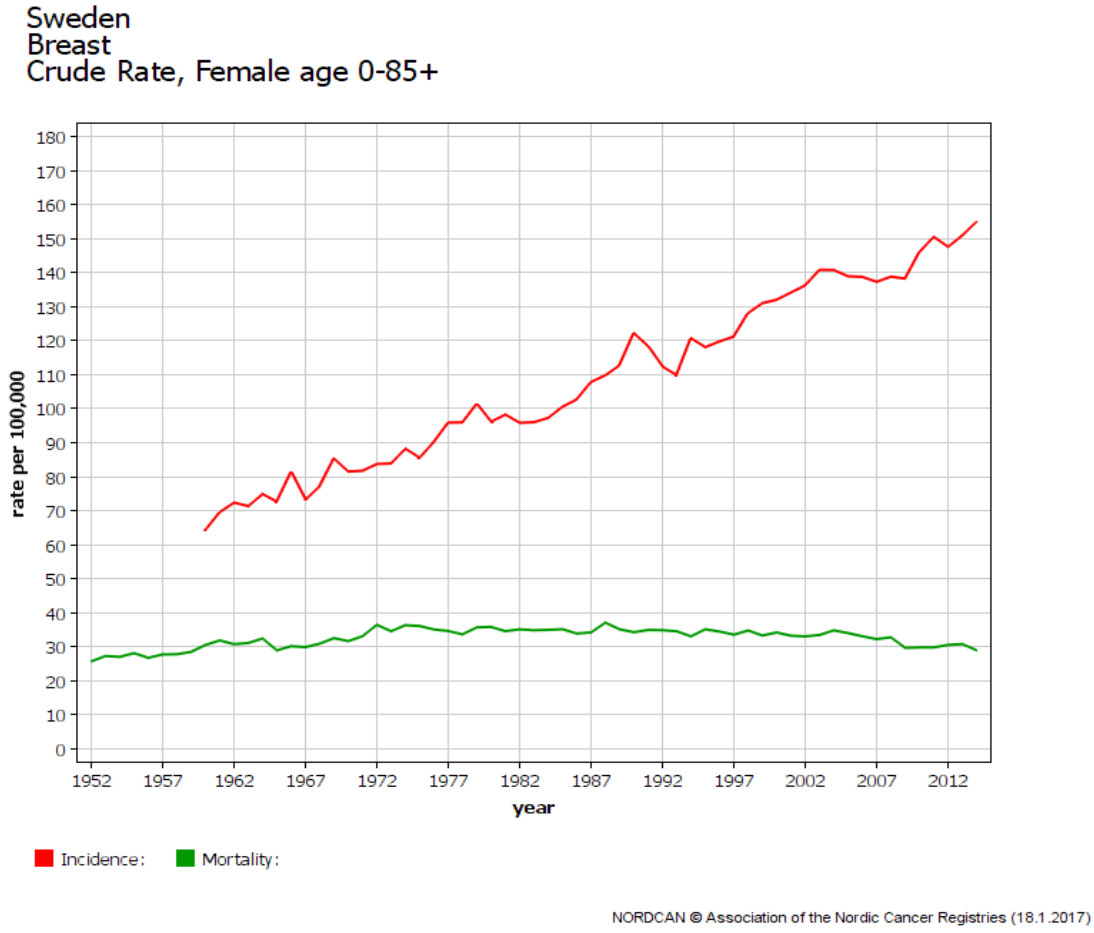

Figure 2 *Time trends in incidence and mortality for female breast cancer in Sweden. Data from NORDCAN (Association of the Nordic Cancer Registries).*

In 2015, 7963 women and 59 men were diagnosed with breast cancer in Sweden [9]. Mortality has slightly decreased over the last two decades, thanks to earlier detection through mammography screening and improved adjuvant treatment. There were 1400 breast cancer-related deaths among women in Sweden in 2011 [10].

The causes of breast cancer are not fully understood but there are several risk factors known to affect the likelihood of developing breast cancer, some more significant than others.

Being of the female sex greatly increases the risk of developing breast cancer. Age also represents a strong risk factor, as approximately 5% of all breast cancer affects women less than 40 years of age [10].

After sex and age, a family history of breast cancer is one of the strongest risk factors. Having a first degree relative with breast cancer doubles the risk, especially if there is low age at onset [11]. Families with three or more close relatives in two generations with breast- or ovarian cancer are regarded as having hereditary breast cancer, accounting for 5-10% of breast malignancies. An inherited disorder in tumor suppressor genes BRCA 1 or BRCA 2 accounts for 2.5-5% of all incidental breast cancer. The life-time risk of developing breast cancer for carriers of these high penetrance gene mutations is 50-80% and for ovarian cancer 25-60% with the higher risks for BRCA 1 mutation carriers [12].

High mammographic density increases the risk four to six-fold compared with women with fatty breasts. Mammographic density changes with age, parity and weight and can be reduced with tamoxifen, which is associated with a better prognosis [13, 14].

Proliferative lesions in the breast, especially if atypia is present can significantly increase the risk of malignant lesions [15].

A high endogenous estrogen level stimulates breast epithelial proliferation and several risk factors are related to reproductive and hormonal factors such as early menarche, late menopause, nulliparity or having the first child after age thirty [16]. Giving birth to many children and breast-feeding each child for more than six months are both protective and reflects the lower incidence in developing countries [7].

Exogenous hormone intake like HRT during the menopause is a well-known risk factor, especially if estrogen is combined with progesterone and taken for a period of five years or more [17]. Oral contraceptives are believed to marginally increase the risk [16].

Extrinsic factors associated with lifestyle also influence the risk. Postmenopausal obesity, low physical activity and high alcohol consumption all increase the risk [18]. Previous chest radiotherapy increases the risk six-fold in young women as seen in survivors after Hodgkin´s lymphoma [19].

2.3 TUMOR CLASSIFICATION

2.3.1 TNM classification

Stage is the most important prognostic factor in cancer and is determined from information on the tumor (T), regional lymph nodes (N) and metastases (M). The TNM system is the most used staging system worldwide and is revised every 6-8 years by the American Joint Committee on Cancer (AJCC) in collaboration with the Union for International Cancer Control (UICC) [20]. Stage is defined at different points in cancer treatment. Clinical stage (pre-treatment stage) is any information about the extent of cancer before initiation of treatment or within four months from diagnosis “prefix c” (cT, cN, cM). Clinical stage is

defined by clinical examination or imaging. Pathologic stage is based on information from histopathological examination of surgically removed tissues at primary definitive surgery "prefix p" (pT, pN, pM).

The post-therapy stage, documents the extent of disease after neoadjuvant systemic therapy initiated before surgical resection, or sometimes without surgical resection. It can either be based on clinical and/or radiological examination (ycT, ycN, ycM) or be based on postoperative pathological findings (ypT, ypN, ypM) [21].

Prognosis and survival is closely related to stage at diagnosis. The 5-year survival rate is almost 100 % in stadium 0-1(T<2 cm and N0), 80 % in stadium II (T<5cm and N1 or T>5 cm and N0), 60% in stadium III (T any size and N 1-3) and only 20 % in stadium IV (any T, any N, M1) [18]. The 5-year relative survival rate (excluding stadium IV) has increased from approximately 65% in the 1970s to 91% in 2015 [9].

2.3.2 Histological grade

Histological grade is also a strong prognostic factor and the morphological grading system described by Bloom and Richardson in 1957 [22], was modified by Elston and Ellis in 1991 to make the criteria more objective. The overall grade (I-III) is derived from the summation of scores from three morphological features; degree of tubular formation (1-3), nuclear atypia (1-3) and mitotic count (1-3). Histological grade together with tumor size and lymph node stage form the Nottingham prognostic index (NPI) which is used to determine prognosis after breast surgery [23].

2.3.3 Histopathologic type

Breast cancer is divided into invasive and non-invasive cancers. The latter is also called carcinoma in situ ("in the same place"), which means it does not invade surrounding tissues.

2.3.3.1 Invasive breast cancer

According to the fourth update of the WHO classification of breast cancer, the terminology for the most common type of breast cancer has changed from invasive ductal carcinoma to invasive carcinoma of no special type (NST). The reason for this is uncertainty of ductal origin and that this does not represent a uniform group of carcinomas. Invasive carcinoma (NST) accounts for 50-80 % of all invasive breast cancer [24]. Among the specific subtypes, lobular carcinoma is the most common form (5-15 %). Immunostaining for the transmembrane protein e-cadherin, which mediates cell-cell adhesion is mostly negative and can help distinguish it from NST [25]. Tubular, medullar, metaplastic, mucinous and papillary carcinomas are all special forms with different prognoses, each occurring in approximately1-2% of all invasive breast cancer [24].

Inflammatory breast cancer (IBC) is a rare but very aggressive entity of breast cancer. The overall five-year survival rate is approximately 30% despite current improvements in therapy. Unfortunately, there are no molecular criteria distinguishing it from non-inflammatory breast

cancer, which would have enabled development of more efficient targeted therapies [26]. The diagnosis is clinical and requires the presence of typical clinical features including; rapid breast enlargement with a diffuse erythema and edema (peau d´orange) of more than a third of the breast skin, and often with a diffuse firmness is in the breast. The presence of tumor emboli within dermal lymphatics is supportive but not sufficient for diagnosis according to the AJCC's cancer staging manual [21].

2.3.3.2 In situ breast cancer

Since the introduction of mammography screening programmes in the mid 1980s, the incidence of ductal carcinoma in situ (DCIS) has increased tremendously. DCIS was previously considered a rare condition diagnosed incidentally at biopsy or symptomatically in the form of a breast lump, nipple discharge or presenting as Paget´s disease of the nipple. Today, DCIS accounts for approximately 10% of all newly diagnosed breast cancer and approximately 20% of all screening-detected breast cancer in Sweden [8], owing to typical microcalcifications often accompanying DCIS visible on mammograms [27].

The 4th edition of theWHO Classification of Tumors of the Breast manual, lists DCIS and lobular neoplasia (LN) as precursor lesions of breast cancer. LN is further subgrouped into lobular carcinoma in situ (LCIS) and pleomorphic lobular carcinoma in situ (PLCIS), the latter being a more aggressive entity. The third lobular entity is the atypical lobular hyperplasia (ALH), separated from the other two by the degree of involvement of the acini [24]. LN is a risk factor for invasive disease in both breasts, but the vast majority of lesions will never progress to invasive breast cancer [28]. Apart from excision with free margins no other treatment is recommended than regular mammography controls [18].

DCIS is a heterogenous pre-invasive form of breast cancer in which the proliferating epithelial cells are confined to the mammary ducts without breaking through the basal membrane and therefore theoretically cannot infiltrate the surrounding tissue and metastasize. The traditional classification was based on growth pattern. However, nuclear grade correlates better with recurrence risk and is more easily reproduced. There are different classification systems based on nuclear grade and most have three groups: low, intermediate and high grade DCIS. In Sweden, grading by Holland is used, which takes into account cell polarization, necrosis and type of calcifications in addition to nuclear grade. The highest grade (grade III) is the most aggressive type [29]. Comedo necrosis is more common in high grade DCIS but is not mandatory [30]. Atypical ductal hyperplasia (ADH) is a proliferative lesion often difficult to distinguish from low grade DCIS on preoperative needle biopsies [24]. The natural history of DCIS is not fully known, but according to a review of studies reevaluating large amounts of breast biopsies initially considered benign and later reclassified as DCIS, the risk of progression to invasive breast cancer over a period of ten years is 14-53% [31]. Local recurrences generally occur at the site of the previous breast excision and invasive recurrences are equally common as in situ. However, young women have a higher risk for a subsequent invasive breast cancer [32].The most important factor in reducing the risk of local recurrence in DCIS is complete excision with clear margins [33].

Microinvasive carcinoma infiltrates across the basement membrane at one or two locations each measuring less than 1mm in its greatest dimension and is usually seen in extensive DCIS especially of high grade [24].

2.3.4 Biomarkers

Imunohistochemical (IHC) analysis of protein gene products; ER, PgR, HER2 and Ki-67, are routinely assessed in breast cancer and are important prognostic but also predictive factors, helping to predict sensitivity to treatment [34]. The Swedish Breast Cancer Group (SweBCG), KVAST (The Swedish National Guidelines for Pathologists working group), Swedish Quality Assurance (SweQA) together with Equalis perform important quality assessment. Reproducibility studies are performed using reference samples distributed to different pathology departments in Sweden to assure quality and comparability between analyses from different laboratories. The reproducibility for ER, PgR and HER2 is high (kappa value 0.8) while it is unsatisfying for Ki-67 (kappa 0.6) [18]. There are national and international collaborations working to improve concordance in Ki-67 scoring [35].

2.3.4.1 Estrogen receptor (ER)

The estrogen receptor (ER) was the first candidate for a specific targeted therapy in breast cancer. Approximately 80-85% of women with invasive breast cancer are ER-positive and more or less sensitive to endocrine therapy [9]. There are two isoforms of ER (ERα and ERβ). ERα is the clinically used isoform and is located in the cell nucleus. The role of ERβ in breast cancer is under much investigation. Its impact on prognosis seems favorable but can differ between treatment groups [36]. The primary ligand is 17-β-estradiol which binds to the receptor and stimulates cell growth by transcription [34]. The most commonly used threshold for ER-positivity has been 10% but in 2010 the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) recommended that ER and Progesteron receptor (PgR) assays with at least 1% positive tumor nuclei should be considered positive [37]. However, in a retrospective study based on 9639 women, those with 1-9% ER-positivity did not seem to benefit from endocrine therapy [38].

2.3.4.2 Progesteron receptor (PgR)

The progesterone receptor (PgR) also exists in two isoforms (PgRα and PgRβ) but distinction is not possible since available antibodies bind to the N-terminal part common to both isoforms. PgR-status is an independent prognostic factor in breast cancer. In a population-based study, PgR negativity was associated with a significantly poorer prognosis in ER-positive node-negative patients receiving endocrine therapy [39]. ER-negative but PR-positive breast cancers are rare and it is not clear if it is a distinct biological entity or a technical artifact since gene expression of PgR is dependent on estrogen, indicating an intact estrogen-ER-signalling pathway [40].

2.3.4.3 HER 2neu/ERBB2

Human epidermal growth factor (HER) 2 is a tyrosine kinase receptor located on the cell surface. Approximately 15% of all breast cancers overexpress HER2 [9]. HER2-status is assessed with immunohistochemistry followed by in situ hybridization if amplification is suspected. Patients with HER2-positive breast cancer have poorer prognosis with shorter overall survival. However, since the development of trastuzumab (Herceptin®), a monoclonal antibody targeted against HER2, the prognosis has improved dramatically and is similar to the prognosis in patients with hormone receptor-positive breast cancer [41].

2.3.4.4 Ki-67

Ki-67 is a monoclonal antibody directed against an antigen (Ki-67 protein) expressed only in proliferating cells. High Ki-67 is a prognostic marker but clinical validation has proved difficult [42]. It is a continuous index of the fraction of proliferating tumor cells. High and low values are reproducible and clinically useful, but there is no standardized cut off level for intermediate values [35, 43]. MIB-1 is another monoclonal antibody directed against the same antigen. It can also be used on formalin-fixed paraffin-embedded sections [44].

2.3.5 Molecular subtypes

Classification of breast cancers into "intrinsic subtypes" based on gene expression patterns using cDNA microarrays and hierarchical clustering can be used to predict survival and how well a cancer will respond to a certain treatment [45, 46]. There are commercially available multigene assays, such as Oncotype DX® or Mammaprint®, that divide tumors into different risk groups based on their gene expression patterns in a number of selected genes. Especially in patients with a good prognosis as in ER-positive node negative breast cancer, these tests can help in deciding who might benefit from adjuvant chemotherapy or not [47]. Since these tests are expensive and not available for most people around the world, surrogate molecular subtypes based on IHC tests (ER, PgR, HER2 and Ki-67) are used to guide treatment decisions. According to the 13th St. Gallen International Breast Cancer Conference [48], the definitions are as follows:

Luminal A-like:	ER pos, PgR pos, HER2 neg, Ki-67 low
Luminal B-like (HER2 negative):	ER pos, HER2 neg and either Ki-67 high or PgR neg
Luminal B-like (HER2 positive):	ER pos, HER2 pos
HER2 positive (non-luminal):	ER neg, PgR neg, HER2 pos
Triple negative (ductal):	ER neg, PgR neg, HER2 neg

2.4 DIAGNOSIS

The investigation of clinically suspicious findings in the breast relies on the combination of physical examination, radiological imaging (mammography and/or ultrasound) and needle

biopsy (fine or core). This clinical work up is usually called triple assessment and has a sensitivity close to 100%. If after triple assessment, any doubt remains, as to whether the lump is malignant or not, a diagnostic surgical breast biopsy is recommended [18]. In Sweden, over 90% of breast cancers are diagnosed preoperatively and a high proportion of patients are discussed at both pre- and postoperative multidisciplinary conferences according to the Swedish National Breast Cancer Registry [9].

In addition to mammography and ultrasound, there are other complementary radiological examinations performed in special situations. Breast MRI (magnetic resonance imaging) is recommended for screening women with inherited susceptibility for breast cancer. In especially young women with dense breasts, MRI has higher sensitivity than mammography and ultrasound. However, the specificity is fairly low, and false-positive findings might lead to diagnostic breast excisions or more extensive surgery. A Swedish prospective multicenter study randomized 440 breast cancer patients to preoperative MRI of the breast (POMB) or not, in addition to conventional imaging. The authors found no significant differences in mastectomy rates between groups. The intervention group had a higher degree of preoperative conversion to mastectomy from breast-conserving surgery but the reoperation rate was significantly reduced in the MRI group compared with the control group [49]. MRI is the recommended imaging tool for response evaluation during and after neoadjuvant systemic therapy. According to a review by Fumagalli et al, ultrasound and mammography should only be used if MRI is not available [50]. In a meta-analysis by Marinovich et al, MRI was found to be more accurate than mammography in detecting residual tumors, although not superior to ultrasound. The authors concluded that further comparative studies are needed [51].

Mammography screening was introduced in Sweden in 1986 and ten years later it was implemented nationally. The National Board of Health and Welfare (Socialstyrelsen) recommends mammography screening between ages 40-74 with an interval of 18-24 months [52]. The recommendations are based on several Swedish randomized trials which have shown reduced mortality rates of 20-25% for women participating in screening programmes [53, 54]. However, there is an ongoing debate where critics claim that the benefits of mortality reduction is prevented by overdiagnosis and overtreatment [55]. The sensitivity of mammography in the screening situation is lower and varies in relation to factors of body weight and how fatty or dense the breasts are [56]. In the screening ages (40-74), approximately 64% of all incidental breast cancers are screening-detected corresponding to just above half of all detected breast cancers in Sweden [9]. Screening-detected breast cancer has a more favorable prognosis due to earlier detection and better tumor biology [57].

2.5 BREAST SURGERY

William Halsted, at John Hopkins Hospital, in 1882 performed the first radical mastectomy removing not only the whole breast but also the underlying pectoralis major and minor muscles together with most of the ipsilateral axillary lymph nodes. This extensive surgery was necessary according to the Halstedian model of cancer progression in which tumors grow

locally, then spread to the regional lymph nodes and finally from there to the rest of the body [58]. The radical mastectomy was the prevailing breast cancer surgery until the 1970s and although it resulted in significantly reduced rates of local recurrence, it was associated with immense morbidity in terms of disfigurement, pain, massive lymphedema, restricted shoulder mobility and sensory loss [59].

In 1948, David Patey described for the first time a modification of Halsted's radical mastectomy in which the pectoralis muscles were spared together with some of the axillary lymph nodes (level III), and despite a much less extensive cancer surgery, the survival rates were comparable [60]. Halsted's model of tumor progression failed to explain the occurrence of distant metastases in women who had been successfully treated with this extensive surgery. Instead the systemic theory developed by Geoffrey Keynes, but fully stated by Bernard Fisher in 1980, won acceptance. According to this theory, local control is not sufficient to impact survival since there is no orderly pattern of tumor cell dissemination [61]. Today breast cancer is thought of as a heterogenous disease, spanning from patients with lymph node metastasis being the only site of dissemination with good prognosis after surgery alone, to others where nodal involvement is only a marker of an already disseminated disease [62, 63]. The modified radical mastectomy developed by Patey, is the method a mastectomy is still performed today.

Breast-conserving surgery (BCS) was introduced in the 1960s and is the primary surgical treatment for small to medium sized breast cancers. Approximately 77% of all newly diagnosed breast cancers less than 3 cm in size are removed with BCS in Sweden [9]. Provided radiotherapy is part of the breast-conserving therapy, survival rates are comparable to mastectomy. This statement is based on several randomized multicenter trials with over twenty years of follow-up [64, 65]. Although local recurrence rates are higher after BCS, the long-term overall survival is comparable [66]. Risk factors for local recurrence are young age, multicentricity and unclear/unknown surgical margins [67]. A consensus guideline based on a meta-analysis from a systematic review of 33 studies including 28162 patients, concluded that positive margins increase the risk two-fold for an ipsilateral local recurrence compared with negative margins. However, wider surgical margins than negative, do not significantly decrease the recurrence risk in invasive breast cancer [68].

2.6 AXILLARY SURGERY

Axillary surgery is primarily performed for nodal staging. Lymph node status is still one of the most important prognostic factors in breast cancer, despite advances in biomarkers and gene expression analysis, and strongly influences therapy decisions [62]. Axillary surgery also provides locoregional control and prevents axillary recurrences. When it comes to survival, earlier randomized studies have not been able to show any convincing survival benefits with axillary dissection in clinically node-negative patients with early-stage breast cancer [69, 70].

Lymph node metastases are divided into macrometastases (pN1) containing tumor deposits larger than 2 mm and micrometastases (pN1mi) with tumor deposits larger than 0.2 mm but not above 2 mm in size. Smaller tumor deposits, up to 0.2 mm, are called isolated tumor cells (ITC) (pN0i+) and these lymph nodes are classified as node-negative (pN0) [71]. There is a relationship between the number of positive lymph nodes and the outcome [72].

Efforts to predict axillary lymph node status based on clinical or biological tumor information has so far not been able to replace surgical nodal staging. In a study investigating 1300 consecutive patients, multivariate analysis found lymphovascular invasion, tumor size, retroareolar or lateral tumor location and multifocality to be the strongest predictors of axillary lymph node metastasis [73]. Neither physical examination [74] nor attempts with different imaging modalities, such as ultrasound with or without fine needle aspiration cytology [75, 76], MRI [77], or positron emission tomography (PET) [78], have so far proven to be sensitive enough to replace surgical nodal staging. However, if no suspicious lymph nodes are detected with ultrasound, more heavy disease burden can be ruled out with relatively high certainty [79].

2.6.1 Axillary lymph node dissection

Axillary lymph node dissection (ALND) levels I and II, has been the standard axillary nodal staging procedure in breast cancer since the introduction of the modified radical mastectomy. With the correct surgical technique, the axillary lymph node yield should be at least ten lymph nodes, which is recommended to avoid misclassification. A large Danish study found that if fewer than ten negative lymph nodes were retrieved, this resulted in incorrect nodal staging with significantly higher recurrence rates and poorer survival outcomes [80].

However, axillary surgery and especially ALND, is associated with potential arm morbidity including seroma formation, nerve damage, paresthesia, chronic pain, impaired shoulder mobility and not to mention lymphedema [81, 82]. Lymphedema can develop secondary to axillary surgery, due to scarring and disruption of lymph vessels resulting in impaired lymph transport capacity. This leads to collections of lymph fluid and proteins in the interstitium that in turn attract more fluid. Eventually the fluid is replaced by adipose tissue [83]. Radiotherapy further increases the risk of lymphedema [84]. The incidence of lymphedema after ALND varies dramatically between studies and is dependent on how lymphedema is defined and measured, as well as the duration of follow-up [85]. In addition, objectively measured lymphedema may differ from self-perceived discomfort and health-related quality of life [86]. After the introduction of mammography screening, the proportion of patients with node-positive breast cancer at diagnosis has decreased to approximately 30% in early-stage breast cancer [87]. In this situation, alternative less extensive surgical staging methods were developed. One of them was axillary sampling, comprising of four or five randomly harvested and analyzed axillary lymph nodes from level I. In Sweden, Ahlgren et al performed a prospective study evaluating 450 patients. The sensitivity was 97% and the negative predictive value was 98% [88]. Despite this and some other studies with rather promising results, axillary sampling has been difficult to implement outside of a clinical trial

setting [1]. There have also been attempts to completely avoid axillary surgery in selected individuals with low-risk, often elderly women with small hormone receptor-positive tumors. In a review and meta-analysis evaluating two randomized controlled trials in elderly women, those women in whom nodal staging was omitted, had significantly more regional recurrences, but overall and disease-free survival was not inferior [89].

2.6.2 Sentinel lymph node biopsy

2.6.2.1 Sentinel lymph node biopsy concept

The sentinel lymph node (SLN) is the first lymph node or nodes that receive(s) lymph fluid from a primary tumor and if this or these nodes are free of tumor cells, the other sequential draining lymph nodes are also free of metastasizing cancer cells with high certainty hence the patient can be spared further nodal dissection. For the breast, the SLN is usually located in the axilla at level I, lateral to the pectoralis minor muscle, but can also be located higher up in the axilla or in the parasternal lymph nodes.

2.6.2.2 Sentinel lymph node history

The term “sentinel node” was first mentioned by Gould et al in 1960 to describe a lymph node situated at the junction between the anterior and posterior facial vein. This lymph node was analyzed intraoperatively and could predict the neck nodal status in patients with cancer in the parotid gland [90]. The sentinel node concept was introduced clinically by Cabanas in 1977 in penile cancer. In 1992 Morton et al used blue dye to identify the draining lymphatic ducts from melanomas [91]. In 1993 Krag described the use of radiolabelled colloid and intraoperative detection of the SLN with a hand-held gamma probe [92]. The year after, Guiliano et al first described blue dye mapping in breast cancer [93]. The SLN technique was introduced in the late 1990s into clinical practice worldwide, as a less extensive nodal staging procedure in breast cancer, with significantly less morbidity compared with ALND. Patients with a negative SLNB could be saved the morbidity associated with ALND [82, 94, 95]. The sentinel lymph node mapping technique is illustrated in Figure 3.

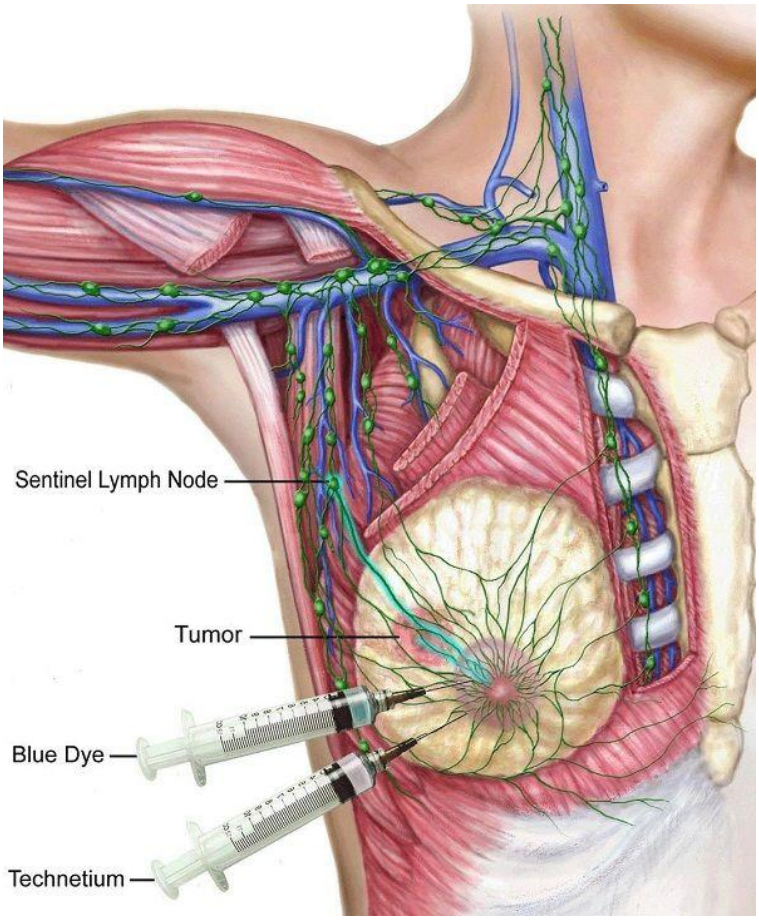

Figure 3 *The sentinel lymph node mapping technique.*
Reproduced with permission from IntraMedical Imaging LLC.

2.6.2.3 Sentinel lymph node mapping

There has been a lot of debate concerning different technical issues and their impact on identification rate (IR) and false negative rate (FNR), such as choice and dose of blue dye and radioactive tracer, particle size and type of the carrier protein for the radioactive tracer, and further where in the breast to inject them and how deep. There is consensus that the combination of blue dye and radiolabelled colloid (dual mapping) gives the best sensitivity for finding nodal metastases in most institutional settings [96]. The blue dyes mostly used are Patent Blue V, Isosulfan Blue and more rarely Methylene Blue. Blue dyes can give rise to anaphylaxis in approximately 1% of procedures [97] and are therefore contraindicated in pregnant women. The standard radioactive tracer is ^{99m}Technetium which has a half-life of six hours that makes it vulnerable in terms of storage and distribution. There has been a worldwide shortage of Technetium and in addition, the radioactive tracer is associated with radiation exposure, hence there is an ongoing search for alternative and preferably nonradioactive substances [98].

A novel technique using a magnetic tracer, supraparamagnetic iron oxide (SPIO), has been evaluated in a number of trials. According to a systematic review and meta-analysis the accuracy is not inferior to the standard technique and there is no radioactivity involved.

However, significantly more SLNs are retrieved and the iron tracer can leave a brown discoloration in the skin at the injection site [99].

The particle size of the carrier protein has implications for how quickly the radioactive tracer is drained to the lymphatic system after injection and how far the radioactive tracer travels in the lymphatics. This also influences how many lymph nodes are mapped before the particles are trapped. The smaller the colloid, the more secondary lymph nodes will be mapped. The optimum colloid size is considered to be 10-100 nm [2]. The most commonly used colloids are nanocolloid albumin, sulfur colloid and antimony trisulphide [100]. The radioactive tracer is injected into the breast preoperatively on the day of surgery or on the day prior. A higher does is required when injecting on the day before surgery. The blue dye is injected in the operating theater when the patient is sedated and safely monitored.

Intraoperatively the surgeon uses a handheld gamma probe to scan the axillary region for radioactivity. The skin incision is made in the area indicating the highest counts with the probe. The SLN definition mostly used is any blue node or node with a blue afferent lymphatic channel, the hottest node and any node with activity >10% of the hottest node and in addition, any palpably suspicious lymph node [101]. However, harvesting more than four SLNs is of no use since it does not impact the false negative rate (FNR) and will instead increase the risk of axillary morbidity and time of surgery [102].

The SLNs are sent fresh to the pathology department for histopathological analysis. With intraoperative analysis the surgeon can proceed with ALND in the same operation if there is a metastasis in the SLNs. Frozen section analysis is the most commonly used method with an overall sensitivity of approximately 75%. The sensitivity for micrometastasis is lower even if IHC staining is performed [103]. Intraoperative analysis is time-consuming for the pathologist and prolongs surgery by approximately 20 minutes. In addition, there is a risk of valuable tumor material being lost during the frozen section procedure [98].

There is a lot of debate and no consensus concerning where in the breast or how deep the radioactive tracer and blue dye should be injected. Following anatomical studies by Sappey et al, stating that the breast is a single ectodermal unit where the deep lymphatics in the breast parenchyma converge through the subareolar plexus on its way to the axillary nodes [3], the site of injection should not have any impact on the lymph drainage pattern. However, deep peritumoral injections drain to a significantly greater extent to extra-axillary SLNs, in particular to the internal mammary nodes in comparison with superficial injections [104]. The number and locations of draining SLNs can be visualized preoperatively with a gamma camera. Planar lymphoscintigraphy was routinely performed following the introduction of the SLN mapping technique. Draining to the internal mammary nodes can be visualized in 5-17% of patients [105, 106]. Although metastasis to the internal mammary nodes has prognostic significance [107], it is unusual with solitary metastasis in these locations and harvesting them is technically demanding, adding extra scars and morbidity that is not negligible. Therefore, most centers prefer to inject the mapping agents superficially in the subareolar plexus and no longer routinely perform preoperative lymphoscintigraphy [108]. It

is instead used as a supplement in special situations [98]. In cases with non-visalization on planar lymphoscintigraphy, hybrid SPECT/CT imaging can be of additional value since the precise anatomical location of SLNs can be visualized [109].

2.6.2.4 Sentinel lymph node accuracy and safety

The SLN technique is not only associated with less morbidity than ALND, it is also a more accurate and sensitive nodal staging procedure. This is due to all SLNs, in contrast to non-SLNs, being serial sectioned and examined with IHC staining with antibodies to cytokeratins [110, 111].

There is a learning curve associated with the performance of SLNB [112]. In 2006 a meta-analysis was published analyzing more than 8000 breast cancer patients from 69 trials treated with SLNB and ALND during the validation period between 1970 and 2003. The IR was overall 96% with a wide range (41-100%) and the overall FNR was 7.3% also with a wide variability (0-29%) between studies [113]. After some years of experience, most studies report FNRs between 5-10 %. Despite this fairly high FNR, local recurrences are rare after a negative SLNB without ALND, even after ten years of follow-up [114, 115]. There are several randomized studies reporting survival data after a negative SLNB not followed by ALND and none of them report inferior survival [116]. The largest study, NSABP B-32, reports after eight years of follow-up, overall survival rates of 90.3% in the SLNB alone group compared with 91.8% in the SLNB and ALND group [117]. SLNB is considered gold standard for nodal staging in early-stage clinically node-negative breast cancer [118] and is currently recommended also in large [119-121] and multifocal/multicentric tumors although the risk of a positive SLNs is higher [122].

2.6.2.5 Current controversies with SLNB

The meticulous examination of SLNs compared with non-SLNs has lead to more and smaller tumor deposits being diagnosed. According to current Swedish National Guidelines from 2014, a complementary ALND should be performed if a micrometastasis is encountered. In this years upcoming new guidelines, ALND is not recommended if BCS is planned. Patients planned for mastectomy can continue to be enrolled into the Swedish prospective cohort study SENOMIC, with the primary endpoint disease-free survival (DFS) after omission of ALND (https://sffb.se) [18]. If there is a micrometastasis in the SLN, there is a 10-20% risk of finding additional non-sentinel node metastases [123]. A recent large retrospective study based on the Surveillance, Epidemiology, and End Results (SEER) database, found that micrometastasis in clinically node-negative women was an independent risk factor for breast cancer mortality [124]. However, a randomized controlled trial (IBCSG 23-01) did not find any significant difference in 5-year DFS between patients with micrometastases in SLNs with or without ALND performed. The majority though had BCS, whole-breast radiotherapy and adjuvant chemotherapy [125].

Also the omission of ALND in clinically node-negative patients with one or two macrometastases in the SLNs is currently being investigated. There is a 40-65% risk of

additional axillary nodal involvement with a positive SLN [126, 127]. The American College of Surgeons Oncology Group (ACOSOG) Z0011 multicenter trial randomized 891 clinically node-negative patients with T1-2 breast cancer and 1-2 SLN macrometastases to complementary ALND or not. All patients had BCS and whole breast irradiation and most patients received adjuvant chemotherapy. Although underpowered, after a median follow-up of six years, survival was comparable between groups. After nine years, local recurrence-free survival was still comparable between groups [128, 129]. Inspired by these results, the Swedish-based international SENOMAC trial (www.senomac.se), was launched in 2015 and randomizes clinically node-negative patients with maximum two SLN macrometastases to a complement ALND or not. The primary outcome measure is breast cancer-specific survival. Since the need for ALND has been questioned, also in patients with node-positive disease, the need for intraoperative analysis of the SLNs has diminished.

DCIS is a pre-invasive condition not able to metastasize. However, DCIS is upgraded to invasive breast cancer in up to 40% of incidental cases on definitive pathology reports [130]. Performing SLNB when mastectomy is indicated for patients with core biopsy-verified DCIS is appropriate since SLNB after a mastectomy is technically impossible. However, if BCS is planned, SLNB is not recommended according to most international guidelines, given the low incidence of positive lymph nodes if signs of potential microinvasion are missing [116, 131]. Despite these recommendations, SLNB is still performed to avoid reoperation and maybe concerns for a less accurate secondary SLN procedure [9].

Prior excisional breast surgery might disrupt lymphatics and disturb lymph drainage from the breast to the axillary lymph nodes. There has been concern that the mapped SLNs will not accurately reflect nodal status in the axilla. Feldman et al reported high FNRs and Borgstein et al low IRs. Both used only radioactive colloid injected peritumorally [132, 133]. Patients with prior breast surgery were therefore excluded from many of the initial prospective studies evaluating SLNB in breast cancer [134]. Later, several studies have shown that SLNB is accurate after a previous breast biopsy [135-137].

There is an ongoing debate on the role and timing of SLNB in the neoadjuvant setting, since also patients with operable breast cancer are candidates for neoadjuvant systemic therapy (NAST), and only half of them have positive lymph nodes at diagnosis and consequently do not benefit from ALND. In addition, current neoadjuvant regimens, including targeted therapies if appropriate, achieve nodal downstaging in as high as 70% of patients [138]. It is controversial whether SLNB should be performed before or after NAST in clinically node-negative patients upfront. If performed before, IR is high but the accuracy has mostly been captured in small single-center studies omitting ALND if the SLNB was negative [139, 140]. SLNB after NAST is associated with lower IR and higher FNR, but only one surgical procedure is necessary [141]. In clinically node-positive patients planned for NAST, SLNB has been evaluated after NAST, in order to make use of nodal conversion. However, FNR has been high in earlier studies ranging from 10-30% and there is a lack of follow- up data [142, 143]. SLNB is not recommended for patients with inflammatory breast cancer (IBC), not

even after nodal downstaging according to ASCO guidelines from 2014 [116]. There are very few studies addressing SLNB in IBC and in both studies identified, the authors conclude that SLNB is unreliable [144] [26, 116].

2.7 SYSTEMIC THERAPY

The main purpose with systemic therapy is to eliminate micrometastatic disaease and circulating tumor cells present at time of diagnosis.

2.7.1 Neoadjuvant (preoperative) systemic therapy

Neoadjuvant systemic therapy (NAST) has been used since the 1970s for the treatment of inoperable locally advanced breast cancer (LABC) and inflammatory breast cancer (IBC). The term LABC includes tumor stage T3 (tumor size>5 cm) or T4abc (tumor of any size with direct extension to the chest wall or skin) but does not include inflammatory breast cancer (T4d) described earlier. LABC also includes nodal stage N2-N3 (fixed or matted ipsilateral axillary, internal mammary or infra/supraclavicular lymph node metastases) [21]. Core-needle biopsy is mandated before the start of NAST. NAST is either systemic chemotherapy or endocrine therapy and is traditionally followed by breast surgery including ALND. After surgery, locoregional radiotherapy has been standard concomitant with endocrine therapy in hormone-sensitive tumors. Also operable breast cancer is increasingly being treated with NAST if adjuvant chemotherapy is indicated. The survival rates are comparable with either regime but the advantages are that more patients can be offered BCS due to downstaging, chemosensitivity can be evaluated in vivo and treatment changed if response is lacking or progress occurs [145, 146]. Additionally, NAST facilitates rapid drug development and approval [147]. With current chemotherapy in combination with targeted anti-HER2 drugs, pathological complete response (pCR) in breast and nodes (ypT0/is/N0) can be achieved in as high as 70% of patients. Patients who achieve pCR have a better survival rate compared with those having residual disease. The association is dependent on tumor subtype and is strongest for patients with HER2-positive and triple-negative tumors [138, 148]. In ER-positive breast cancer, neoadjuvant endocrine therapy may be an option, especially in frail elderly women. Aromatase inhibitors are more efficient than tamoxifen. If the disease is stable, treatment duration can be life-long if surgery is not suitable or desirable [149]. In 2015, neoadjuvant instead of adjuvant chemotherapy was given to 19% (range 4-26%) of newly diagnosed Swedish breast cancer patients [9].

2.7.2 Adjuvant chemotherapy

Chemotherapy has been in use since the 1960s, initially in patients with breast cancer recurrence[150]. Adjuvant therapy is given in addition to primary surgery with curative intent to patients with regional lymph node metastasis but also to node-negative patients with ER-negative tumors or if other risk factors such as young age, high grade, high proliferation or lymphovascular invasion are present [18]. The surrogate molecular subtypes are, besides nodal status, the most important factors for therapy planning. A meta-analysis on behalf of the Early Breast Cancer Trialists´ Collaborative Group (EBCTCG) including 123 randomized

trials and 100.000 patients, showed that adjuvant polychemotherapy with high-dose antracyclines are more efficient than earlier CMF-combinations although associated with more cardiac toxicity. The addition of taxanes to anthracyclines was even more efficient and 10 year breast cancer mortality could be reduced by about one third. The relative benefit with chemotherapy was not dependent on age, tumor size, nodal stage, grade, ER-status or tamoxifen use. However, the absolute gain was proportional to an individual´s risk without chemotherapy [151].

2.7.3 Targeted therapy

HER2-positive patients left untreated have a worse prognosis. Trastuzumab given together with chemotherpy in early breast cancer, improves survival significantly (hazard ratio 0.66) and 1 year of trastuzumab is now standard in HER2-positive breast cancer larger than 5 mm in size [35, 152] but the risk for congestive heart failure is substantial [153]. In the neoadjuvant setting, HER2-positive patients who receive trastuzumab in combination with pertuzumab and taxanes, achieve significantly higher pCR rates compared with chemotherapy and trastuzumab alone [154, 155].

2.7.4 Adjuvant endocrine therapy

Approximately 80% of breast cancer patients have ER-positive tumors, the growth of which is stimulated by hormones. Adjuvant endocrine therapy either blocks or lowers the circulating endogen hormone levels, thereby reducing both local and distant recurrences. They are administered following chemotherapy since they decrease the proportion of proliferating cells, making the tumor less sensitive to chemotherapy. In ER-positive patients, five years of tamoxifen reduces recurrence rates by about half and breast cancer-specific mortality by one third during the first 15 years after the start of treatment [156]. Extending tamoxifen to ten years can further reduce recurrence rates and DFS but compliance is a problem that needs attention [18]. Aromatase inhibitors are first choice in postmenopausal women with high-risk tumors owing to more efficient local control compared with tamoxifen, even though the effects on mortality are uncertain [157].

2.8 ADJUVANT RADIOTHERAPY

According to international guidelines from the European Society of Breast Cancer Specialists (EUSOMA) and the American Society for Radiation Oncology (ASTRO), radiotherapy is indicated if the local recurrence risk exceeds 20% within 10 years. This applies for women after BCS, after mastectomy if the tumor is larger than 5 cm, and in case of four or more positive axillary lymph nodes [158].Whole breast irradiation is part of the therapy for patients with invasive breast cancer having BCS and reduces the local recurrence risk by half and breast cancer death rate by about a sixth [159]. In patients with a very low risk of local recurrence, radiotherapy may be omitted. There is since 2005 an ongoing Swedish national study (“Strålkohortstudien”), evaluating the omission of radiotherapy after BCS in a cohort of patients older than 65 years with small tumors [9]. Also in DCIS, long-term follow-up show that radiotherapy after BCS approximately halves the local recurrence risk [160-163].

However, the survival benefit is minimal since survival in DCIS is comparable to the background population [164]. In the updated meta-analysis by EBCTCG from 2014, it is concluded that radiotherapy reduces both locoregional recurrence rates and breast cancer mortality also in women having modified radical mastectomy and 1-3 positive lymph nodes [165]. Swedish National Guidelines recommend locoregional radiotherapy in this scenario but do not routinely include the internal mammary lymph nodes [18]. However, a large multicenter trial randomly assigned patients with node-negative medially or centrally located breast cancers or node-positive laterally located breast cancers, to regional nodal irradiation including internal mammary and medial supraclavicular lymp nodes or not, and reported after median 10 years of follow-up improved DFS and distant DFS although overall survival was not significantly improved [166].

3 AIMS OF THE THESIS

The overall aim of this thesis was to investigate sentinel lymph node biopsy (SLNB) and its current role in breast cancer treatment in with a focus on current controversies and limitations within different clinical settings and to examine whether our results have implications on future treatment indications. Specifically the aims were as follows:

3.1 PAPER I

To investigate the incidence of axillary surgery and node-positivity in patients with pure ductal carcinoma in situ according to the final pathology report. The secondary aim was to investigate whether additional tumor sectioning would reveal overlooked occult microinvasion, possibly obviating the need for SLNB.

3.2 PAPER II

To investigate the impact of prior diagnostic excisional surgery on the number and locations of sentinel lymph nodes, before compared with after breast surgery, evaluated with hybrid SPECT/CT imaging.

3.3 PAPER III

To investigate the accuracy and clinical relevance of SLNB performed before neoadjuvant systemic therapy (NAST) in clinically node-negative patients with breast cancer within a multicenter setting.

3.4 PAPER IV

To investigate the feasibility and accuracy of SLNB performed after NAST in clinically node-positive patients with breast cancer within a multicenter setting and evaluate whether axillary lymph node dissection can be omitted in case of nodal downstaging.

4 PATIENTS AND METHODS

4.1 PATIENTS

4.1.1 Paper I

A retrospective registry study investigating the incidence of SLN metastasis in patients with a postoperative diagnosis of pure DCIS (ductal carcinoma in situ). The data source used was the web-based Swedish National Breast Cancer Registry (INCA) [9], which combines mandatory reporting to the Swedish Cancer Registry [8], with voluntary reporting to the Breast Cancer Registry [9]. Double reporting can thus be avoided. Nearly100 % of all newly diagnosed breast cancers are reported, and the primary data completion rate was 97% in 2015. From the registry, all patients primarily operated for a pure DCIS according to the the final pathology report in the period January 1, 2008 to December 31, 2009 were retrieved. For practical reasons validation of the register was performed in 15% of patients registered in the Stockholm area. In these 40 individuals, 13 out of 1160 variable fields were incorrect (1.1%). However, in the registered data from outside Stockholm, incongruent data and missing values were more common. It was therefore necessary to review the medical records and pathology reports for 338 individuals, correcting and validating the extracted data.

4.1.2 Paper II

A prospective study evaluating lymph drainage alterations from the breast to the axillary lymph nodes after prior diagnostic breast surgery. Patients were eligible for enrollment if they were scheduled for unilateral excisional breast surgery or diagnostic BCS for a most likely benign tumor. Indications for surgery were growing fibroadenomas, papillomas with or without nipple discharge, inconclusive findings after triple assessment and the patient's own choice to remove a benign lump. The surgery was performed at Södersjukhuset between December 2010 and December 2014. Exclusion criteria were planned bilateral surgery, pregnancy, physical or psychological inability to participate and linguistic difficulties.

4.1.3 Paper III & IV

A prospective multicenter trial evaluating the accuracy and clinical relevance of SLNB within the neoadjuvant setting. The trial opened in October 2010 and initially there were 17 recruiting hospitals, but owing to a slow inclusion rate, three more hospitals were invited after ethical approval. All patients scheduled for NAST with biopsy-proven invasive breast cancer were eligible. Depending on their clinical nodal status at diagnosis, patients were enrolled into two different arms of the trial. In the first arm (paper III), clinically node-negative patients were prospectively enrolled at thirteen recruiting hospitals. In the second arm (paper IV) patients with T1-4d biopsy-proven clinically node-positive breast cancer were enrolled at ten recruiting hospitals. The exclusion criteria were allergic reactions to blue dye or radioactive tracer and the inability to give informed consent. In paper III, patients with

inflammatory breast cancer (IBC) were excluded in addition. The whole trial closed in December 2015 after having reached its accrual goal for paper III.

4.2 METHODS

4.2.1 Paper I

A case-control study was performed in choosing two matched SLN-negative individuals for each patient with tumor deposits in the SLNs. The matching criteria were nuclear grade, tumor extent and closest age. All remaining archived paraffin blocks from the primary tumors were retrieved from the 14 corresponding pathology departments after approval from the local biobank coordinator. Additional sectioning of the tumor blocks including large sections if available, was performed to expand the examined tissue as follows: From the surface of the block (level 1) three sections were cut and then the block surface was cut down 150 µm and three sections 4-4.5 µm each were saved (level 2). Then the block was cut down an additional 150 µm and three more sections with the same thickness were again collected (level 3). One section from each of the three levels was stained with haematoxylin and eosin and examined by a senior pathologist blinded to all tumor and patient information. If microinvasion was suspected, IHC staining with myoeptielial markers p63 and calponin was performed on the two remaining sections from that same level. Another experienced pathologist confirmed all areas with microinvasion. SLN metastasis was classified according to the AJCC staging manual.

4.2.2 Paper II

One week prior to surgery and approximately six weeks postoperatively, patients were examined with planar scintigraphy and SPECT/CT integrated dual head gamma imaging camera (Siemens Symbia T16, Erlangen, Germany) with low-energy high-resolution collimators. Each breast was injected subcutaneously inferior to the areola with 0.4 ml of ^{99m}Technetium labeled nanocolloid. The non-operated breast served as a control evaluating reproducibility with SPECT/CT imaging performed repeatedly. Each procedure started with planar images with lymphoscintigraphy one hour after injection with the patient in the prone projection with her hands up behind the head. The images were reviewed and if no tracer uptake was evident on either side, repeated lymphoscintigraphy was performed two hours after injection. SPECT/CT imaging was performed whenever a hot sentinel node was detected on planar images. SPECT acquisition was performed with 64 projections over an angle of 360 º and the projections were reconstructed with Hybrid Recon™ (Hermes Medical Solutions). CT images were subsequently taken with the patient in the same position, images reconstructed and optimized for soft tissue and with 5 mm slice thickness. SPECT and CT images were then fused and reviewed in a Hybrid Viewer™ (Hermes Medical Solutions). The whole procedure was repeated six weeks postoperatively. Images were reviewed by an experienced breast surgeon and a senior resident in radiology, and the reviewers had no knowledge of which breast was the operated one. SPECT/CT images prior to and following

surgery were compared in three projections (sagittal, coronal and transaxial). A radioactive SLN in the left axilla is visualized with SPECT/CT imaging and displayed in Figure 4.

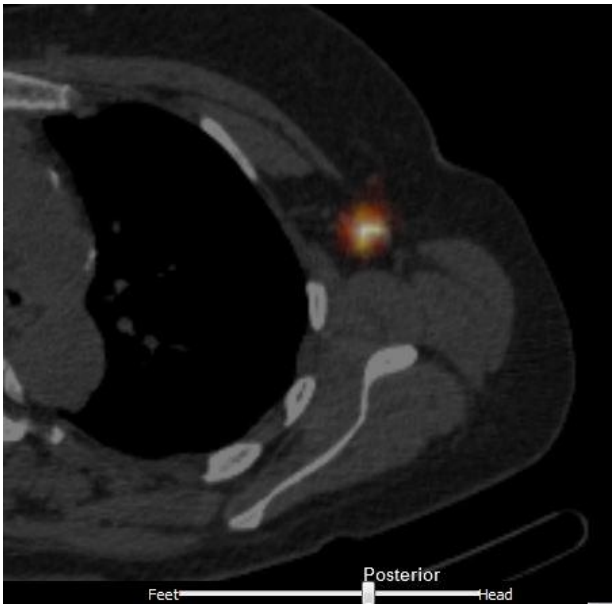

Figure 4 *Hybrid SPECT/CT imaging visualizing a radioactive SLN in the left axilla of a study patient (transaxial projection).*

All operated and non-operated breasts were reviewed separately and analyzed. Results were classified as follows:

Total concordance: the same number and locations of SLNs were recorded postoperatively, but also further SLNs in addition to those recorded preoperatively were included here.

Partial concordance: at least one SLN was recorded in the same location postoperatively, but an overall lower number of SLNs were recorded.

Discordance: no SLNs were postoperatively found in the same location as recorded preoperatively or there were no visible radioactive SLNs either pre-or postoperatively.

Breast sides with no visible SLNs neither pre- nor postoperatively were excluded from concordance analysis.

BMI was subdivided into normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) and obese (30.0 kg/m^2 and over). The radiological size of the lesion was primarily based on mammographical measurements. For lesions not detectable on mammography, the sonograhical dimensions were used.

4.2.3 Paper III

All patients in the trial had biopsy-proven invasive breast cancer. Histological grade and type including biomarkers were assessed on pre-therapy core biopsy findings. Clinical tumor size at diagnosis was based on mammographical measurements, but in a few cases mammographical size was not possible to determine and sonographical measurements were used instead. Regional lymph nodes were examined with ultrasound and if suspicious lymph nodes were encountered, fine needle aspiration cytology (FNAC) was performed. Clinically node-negative patients were eligible for this arm of the trial. SLNB was performed before the start of NAST. A repeat SLNB after NAST was encouraged but not mandatory. After NAST, ALND was performed in all patients together with breast surgery irrespective of the result of the SLNB upfront. A flow chart of the trial is presented in Figure 5.

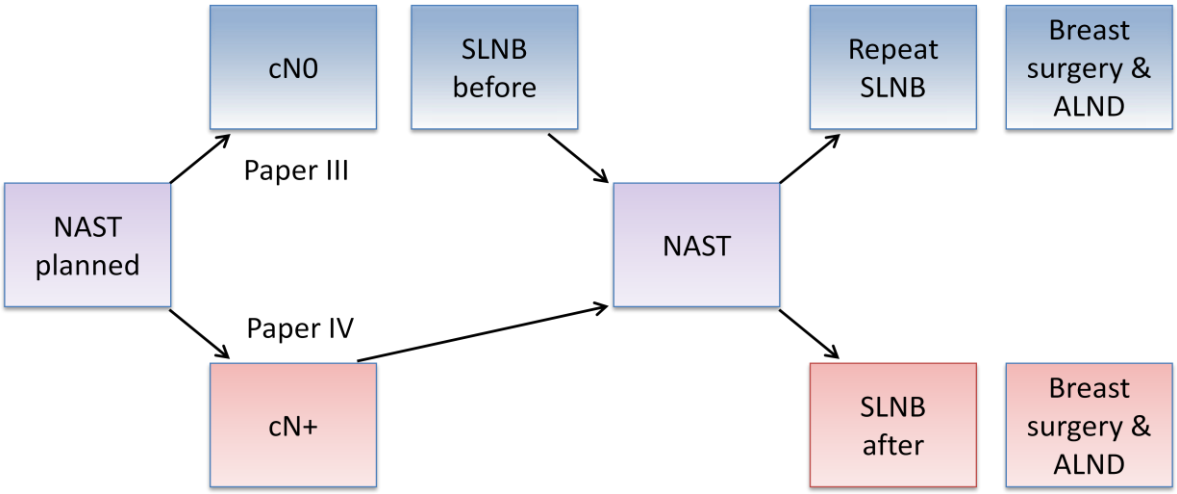

Figure 5 *Flow chart of the Swedish multicenter trial evaluating SLNB in NAST. SLNB sentinel lymph node biopsy, ALND axillary lymph node dissection, NAST neoadjuvanat systemic therapy, cN0 clinically node-negative, cN+ clinically node-positive.*

Preoperative lymphoscinitigraphy was optional. Lymphatic mapping was performed with ^{99m}Technetium-labeled nanocolloid injected in the breast on the day of surgery or the day before. Intraoperatively a handheld gamma probe was used to identify radioactive lymph nodes alone or mostly together with Patent Blue V® dye (Guerbet, Paris, France) injected in the breast approximately ten minutes before skin incision. A SLN was defined as any blue node or node with afferent blue lymphatics, the hottest node and any node with gamma count higher than 10% of the hottest node and finally, suspicious nodes on digital exploration. Intraoperative SLN analysis was not performed before NAST, but was allowed in repeat SLNB after NAST. Both neoadjuvant chemotherapy and endocrine therapy were allowed. Standard chemotherapy was given according to regional guidelines or within ongoing study protocols. Endocrine therapy consisted of aromatase inhibitors. Anti-HER2 therapy in HER2-positive patients was either single or double blockage.

Response evaluation was performed by comparing clinical and radiological findings in breast and regional lymph nodes at diagnosis and before definitive surgery with information extracted from medical records and pathology reports. Clinical response was evaluated

according to UICC criteria [167]. Radiological response was evaluated with mammography and or ultrasound and assessed by a breast radiologist according to the Response Evaluation Criteria In Solid Tumors (RECIST)-criteria [168]. Pathological response was assessed by a breast pathologist, the principal investigator or the local study coordinating breast surgeon after reviewing pathology reports. The response was classified according to Sataloff et al [169]. The different classifications used for response evaluation are presented in Table 1.

Table 1 Different classifications used for response evaluation of NAST in papers III and IV.

	Complete response	**Partial response**	**No change**	**Progress**
Clinical response in tumor (ycT)	The disappearance of all known disease	50% or more decrease in total tumor load	A 50% decrease in total tumor size cannot be established nor an increase of 25%	25% or more increase in size of one or more measurable lesions
Radiological response in tumor (ycT)	The disappearance of all known disease	30% or more decrease in the sum of the longest diameter in target lesions	A 30% decrease in the sum of the longest diameter in target lesions cannot be established nor an increase of 25%	25% or more increase in the sum of the longest diameter in target lesions
	Sataloff A	Sataloff B	Sataloff C	Sataloff D
Pathological response in tumor (ypT)	Total or near total therapeutic effect (T-A)	>50% therapeutic effect but less than total or near total (T-B)	<50% therapeutic effect, but effect evident (T-C)	No therapeutic effect (T-D)
Pathological response in nodes (ypN)	Evidence of therapeutic effect, no metastatic disease (N-A)	No nodal metastasis or therapeutic effect (N-B)	Evidence of therapeutic effect but nodal metastasis still present (N-C)	Viable metastatic disease, no therapeutic effect (N-D)

Breast surgery was either BCS or mastectomy. Axillary lymph node dissection levels I and II was performed in all patients.

The histopathological examination was according to national guidelines as stated in the KVAST document [170]. All SLNs were fixed in formalin and sliced at 2 mm intervals and embedded in paraffin. Each paraffin block was then serial sectioned at three 200 µm levels and each level stained with haematoxylin and eosin. IHC staining with cytokeratin was not mandatory but was recommended if no tumor deposits were detected at that level. Non-SLNs were also handled according to national guidelines. Postoperative stage classification (ypTNM) and SLN metastasis classifications were based on the 7th edition of the AJCC staging manual. No residual invasive cancer but residual in situ carcinoma was considered pathological complete response (ypT0/is) [171]. The presence of isolated tumor cells ypN0(i+), was not defined as nodal pCR [21] .

True-negative: patients with negative SLNs and negative non-SLNs.

True-positive: patients with positive SLNs and positive or negative non-SLNs.

False-negative: patients with negative SLNs and at least one positive non-SLN.

Identification rate (IR): the number of patients with a successfully identified SLN divided by the number of patients in whom an SLNB was attempted.

False negative rate (FNR): The proportion of patients with a false-negative SLN before NAST divided by all node-positive patients. In SLNB after NAST, FNR was defined as the proportion of patients with a false-negative SLN after NAST divided by all patients with an identified SLN after NAST and at least one positive axillary lymph node after NAST.

Accuracy: the proportion of patients with a true-positive or true-negative SLN out of all patients with successfully identified SLNs.

Sensitivity: the proportion of patients with a positive SLN out of all node-positive patients.

Specificity: the proportion of patients with a negative SLN out of all patients with only negative lymph nodes.

Positive predictive value: the probability that patients with positive SLNs have positive non-SLNs.

Negative predicitive value: the probability that patients with negative SLNs have negative non-SLNs.

4.2.4 Paper IV

In paper IV similar methods as outlined for paper III were utilized but with a set of amendments. In the second arm of the trial, patients with T1-4d stage breast cancer planned for NAST with biopsy-proven node positive disease were eligible. SLNB was attempted in all patients after NAST together with a mandatory ALND. Clinical node negativity after NAST was not a requirement for SLNB to be attempted. The magnetic tracer SPIO was allowed alone or in combination with blue dye or radioactive tracer. Intraoperative analysis of SLNs after NAST was allowed but was not mandatory. The trial is registered with the identifier NCT02031042 at clinical.trials.gov.

4.3 STATISTICAL ANALYSIS

4.3.1 Paper I

Factors affecting the decision to perform SLNB were analyzed by a univariable logistic regression model for each independent factor separately followed by a multivariable logistic regression model. Results were given as odds ratios (OR) with corresponding 95% confidence intervals (CI). Only variables known preoperatively were entered in the regression analysis. Univariable and multivariable logisitic regression analyses were also performed with SLN metastasis (N1 according to TNM classification) as the dependent variable. The agreement of clinical estimates of tumor size in case of a palpable tumor, to final pathological tumor size was assessed using Cohen's kappa. The κ value corresponds with poor <0.00, slight 0.00-0.20, fair 0.21-0.40, moderate 0.41-0.6, substantial 0.61-0.80 and almost perfect >0.80 agreement. The variable hospital volume, was subdivided after visual binning into low

(≤ 10 SLNB procedures per year), intermediate (11-29 procedures) and high volume (≥30 procedures). P<0.05 was considered statistically significant. The statistical software IBM SPSS Statistics Version 21.0 (Armonk, NY; USA) was used for all analyses.

4.3.2 Paper II

The comparison of groups according to SLN concordance in operated breasts, SLN visualization per procedure and the distribution of non-visualisation between operated and non-operated procedures was performed after exploring the data distribution. For comparison of non-parametric continuous data, the Mann-Whitney U test was applied, for non-parametric categorical data, Pearson's Chi-tests were applied and Fisher's exact test was used for low case numbers. For the comparison of parametric continuous data, the independent two samples t-test using equal variance was used. P<0.05 was considered statistically significant. IBM SPSS Statistics Version 22.0 (Armonk, NY, USA) was used for all analyses.

4.3.3 Paper III & IV

A sample size calculation was performed prior to initiation of the trial for paper III only, since the aim of paper III was primary aim for the wole trial. With an estimated 50% of all clinically node-negative patients having a positive SLNB, and a proposed sample size of 200 patients, estimation of the FNR in SLNB before NAST was based on 100 patients. If assuming a true FNR in the population of 8%, a power of 80% will be achieved with reported confidence intervals (CI) of +/- 7 percentages. Comparison of false-negative to true-positive and true-negative SLNs before (paper III) or after (paper IV) NAST was performed after exploring the data distribution. For comparison of non-parametric continuous data the Mann-Whitney U test was used and for non-parametric categorical data, the Fisher's exact test was used. A p-value of <0.05 was considered statistically significant. The statistical software program IBM SPSS Statistics for Windows Version 23.0 (Armonk, NY, USA) was used for all analyses.

4.4 ETHICAL CONSIDERATIONS

4.4.1 Paper I

This was a registry study so consent from the study participants was not necessary. Ethical permission for review of medical records and pathology reports to complement and correct incongruent data, as well as permission for additional sectioning of tumor tissue blocks, was sought and approved. Two patients of the matched cases in which the additional sectioning of the tumor blocks revealed occult microinvasion, were not aware that their tumor tissue blocks were reviewed and we decided not to inform them, since several years had passed since their primary surgery and change in therapy was not relevant.

4.4.2 Paper II

Written informed consent was obtained from all study subjects. Due to slow accrual, patients were eventually paid to cover loss of income due to absence from work for each of the two

SPECT/CT imaging procedures, which took a couple of hours in time each to complete. Before each imaging procedure, an amount of 30 MBq radioactive nanocolloid was injected in each breast below the areola. The total radiation dose for both procedures including SPECT/CT imaging was estimated to 4.6 mSv, which is equivalent to four years of background radiation. Approval from both the Regional Ethics Committee at Stockholm County and the Radiation Protection Committee at Karolinska University Hospital Huddinge was attained.

4.4.3 Paper III & IV

All study subjects were prospectively enrolled and informed about the trial. Written informed consent was mandatory before trial eligibility. SLNBs were performed before NAST in paper III and consequently an extra surgical intervention was needed with risk of delaying the start of NAST. However, the benefit was that half of the patients were informed of negative SLNs within a couple of weeks. This information would otherwise not have been available until after definitive surgery. Additionally, axillary radiotherapy could be omitted in patients with negative SLNs upfront. On the other hand, those being both clinically and pathologically node-negative at diagnosis with operable breast tumors, could have been spared the morbidity of the mandatory ALND, if primary surgery would have been performed instead. However, there are other advantages with NAST as previously mentioned, which also applied to the study subjects.

In paper III SLNB was performed both before and in some patients repeatedly after NAST. Lymphatic mapping was performed on both occasions with radiolabeled nanocolloid injected into the breast together with Patent Blue V dye in most patients. The radiation dose associated with each procedure was 40 MBq, if injected the same day, or the double the amount if administered the day before. The radiation exposure from each injection was less than 1 mSv, approximately equivalent to one year of background radiation. According to the literature, the radiation exposure associated with SLNB is minimal [100]. The blue dye used in the trial, was injected after the patient was anaesthetized, thereby avoiding the pain from the injection. In addition the patient was securely supervised, which is important since the blue dye can cause anaphylactic reactions in 0.5-1% of patients [97]. Especially if BCS is performed, a blue discoloration in the skin can be left in place for months, but eventually disappears. In paper IV, a few patients were mapped with the magnetic tracer SPIO, which can leave a brown discoloration in the skin. This discoloration may still be present after 15 months in approximately 8% of patients according to Karakatsanis et al [172].

5 RESULTS

5.1 PAPER I

Initially, 1325 patients with a postoperative diagnosis of pure DCIS were identified, but after review of selected medical records and pathology reports, 52 patients were excluded leaving 1273 patients for the final analysis. A flow chart is displayed in Figure 6.

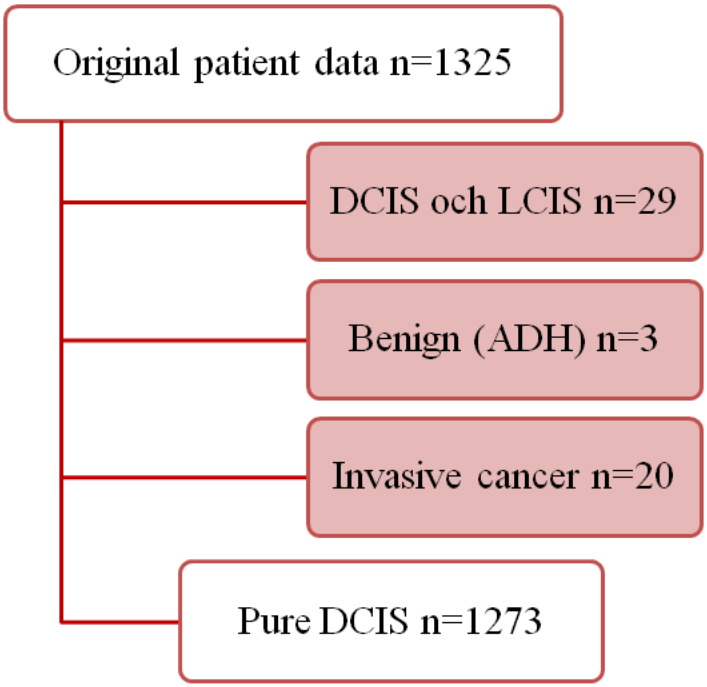

Figure 6 *Flow chart for the exclusion of cases in paper I.*
DCIS ductal carcinoma in situ, LCIS lobular carcinoma in situ, ADH atypical ductal hyperplasia.

The median age of the patients was 60 years (range 26-92). Preoperative tumor size estimated clinically was in agreement with the postoperative histopathological report in 60.1% (196/326) of cases with palpable tumors (κ=0.33). Clinicopathological characteristics, including hospital volume are displayed in Table 2. SLNB was performed in 59.2% (753/1273) of patients. In addition, axillary sampling was performed in five and ALND in 19 patients without tumor deposits in the retrieved lymph nodes, thus 61.0% (777/1273) had some kind of axillary surgery. Five out of 753 (0.7%) patients with SLNB performed, had SLN metastasis (pN1 or pN1mi). Three of these five patients also had ALND with no additional non-SLN metastases found. In addition, six patients had isolated tumor cells in the SLNs (pN0(i+)). Further details on the eleven patients with tumor deposits in their SLNs are displayed in Table 3.

Table 2 Main characterisitics of the study population in paper I.

	No. of patients* (n=1273)
Hospital volume	
Low	214 (16.8)
Intermediate	772 (60.6)
High volume	287 (22.5)
Age (years)	
Median (range)	60 (26-92)
≤50	297 (23.3)
51-70	766 (60.2)
≥70	210 (16.5)
Final breast surgery	
BCS	762 (59.9)
Mastectomy	509 (40.0)
Unknown	2 (0.2)
Multidisciplinary conference	
No	149 (11.7)
Yes	1089 (85.5)
Unknown	35 (2.7)
Method of diagnosis	
Histopathological	715 (56.1)
Cytological	501 (39.3)
Unknown	57 (4.5)
DCIS size pathological (mm)	
Median (range)	20 (1-150)
≤20	671 (52.7)
21-50	413 (32.4)
≥51	139 (10.9)
Unknown	50 (3.9)
Nuclear grade	
1	158 (12.4)
2	384 (30.1)
3	513 (40.3)
Unknown	218 (17.1)

*With percentages in parentheses unless indicated otherwise.
DCIS ductal carcinoma in situ, BCS breast-conserving surgery.

Additional tumor sections were reviewed in the eleven patients with tumor deposits in the SLNs and in 21 of 22 matched controls with available tumor blocks. A median of 8 blocks were available per patient (range 1-21). In one out of 11 women (9%) with SLN tumor deposits, and in two out of 21 matched control patients without tumor deposits, occult tumor invasion was found.

In univariable and multivariable logistic regression analysis, none of the examined variables age, screening-detected, palpability, method of detection, histopathological tumor size or nuclear grade, were significantly associated with SLN metastasis. Predictors of SLN metastasis are displayed in Table 4.

Table 3 Characteristics of the 11 patients with pure DCIS and SLN tumor deposits.

Pat no.	Final breast surgery	Tumor extent (mm)	Nuclear grade	SLN met	Number of SLNs	Axillary met (number)	Axillary lymph nodes (number)	Final axillary staging procedure
58	BCS	18	3	pN1	1	1	13	clearance
50	mastectomy	.	3	pN1	3	1	13	clearance
80	mastectomy	50	3	pN1mi	3	1	5	sampling
53	mastectomy	50	3	pN1mi	2	1	15	clearance
70	mastectomy	35	2	pN1mi	2	1	2	SLNB
61	mastectomy	50	2	pN0(i+)	2	0	3	SLNB
54	mastectomy	40	3	pN0(i+)	1	0	1	SLNB
54	mastectomy	50	3	pN0(i+)	1	0	6	clearance
44	mastectomy	40	3	pN0(i+)	3	0	7	sampling
42	BCS	12	3	pN0(i+)	2	0	5	sampling
33	mastectomy	55	2	pN0(i+)	3	0	3	SLNB

pN1: macrometastasis >2 mm, pN1mi: micrometastasis >0.2 but ≤ 2mm, pN0(i+): isolated tumor cells ≤0.2 mm, BCS breast-conserving surgery, SLN sentinel lymph node, SLNB sentinel lymph node biopsy, DCIS ductal carcinoma in situ

Table 4 Predictors of SLN metastasis in pure DCIS.

Predictors of SLN metastasis N1	Univariable			Multivariable		
	OR	CI	P-value	OR	CI	P-value
Age						
≤ 50 yrs	ref					
51-70 yrs	1.25	0.13-12.10	0.847			0.996
≥71 yrs	1.86	0.11-30.10	0.661			1.000
Screening-detected						
No	ref					
Yes	0.27	0.05-1.62	0.152	0.61	0.05-7.36	0.698
Palpable tumor						
No	ref					
Yes			0.993			0.993
Method of diagnosis						
Histopathological	ref					
Cytological			0.994			0.994
Pathological T size						
pT1	ref					
pT2	3.35	0.35-32.36	0.297	1.86	0.15-22.85	0.627
pT3	0.00		0.997			0.997
Nuclear grade						
Grade 1	ref					
Grade 2			0.998			0.997
Grade 3			0.998			0.997

OR odds ratio, CI 95% confidence interval, SLN sentinel lymph node, pT1 tumor size1≤20 mm, pT2 tumor size 21-50 mm, pT3 tumor size>50 mm.

Table 5 Predictors of SLNB in preoperatively diagnosed DCIS.

	Univariable			Multivariable		
Predictors of SLNB	**OR**	**CI**	**P-value**	**OR**	**CI**	**P-value**
Hospital volume						
Low	ref					
Intermediate	1.33	0.98-1.80	0.067	1.10	0.70-1.70	0.698
High	1.60	1.12-2.30	0.010*	0.94	0.56-1.57	0.806
Age						
≤ 50 yrs	Ref					
51-70 yrs	0.83	0.63-1.10	0.188	0.71	0.48-1.06	0.094
≥71 yrs	0.53	0.37-0.77	0.001**	0.43	0.25-0.72	0.001**
Type of breast surgery						
BCS	Ref					
Mastectomy	4.25	3.29-5.49	0.000**	4.26	2.99-6.07	0.000**
Multidisciplinary conference						
No	Ref					
Yes	1.47	1.04-2.07	0.029*	1.57	0.97-2.53	0.066
Screening-detected						
No	ref					
Yes	1.52	1.20-1.93	0.001**	2.37	1.58-3.54	0.000**
Method of diagnosis						
Histopathological diagnosis	ref					
Cytological diagnosis	3.875	2,99-5.01	0.000**	5.30	3.74-7.50	0.000**
Palpable tumor						
Not palpable	ref					
cT1	2.23	1.62-3.10	0.000**	2.54	1.62-3.98	0.000**
cT2	3.86	2.24-6.63	0.000**	2.67	1.36-5.213	0.004**
cT3	5.68	1.97-16.41	0.001**	2.97	0.80-11.01	0.103

* p< 0.05, ** p< 0.01. OR odds ratio, CI 95% confidence interval, BCS breast-conserving surgery. cT1≤20 mm, cT2 21-50 mm, cT3>50 mm.

On univariable logistic regression analysis, factors significantly associated with the decision to perform SLNB were hospital volume, age, type of breast surgery, discussion at a multidisciplinary cancer conference, detection by screening, method of diagnosis and preoperatively assessed clinical tumor size.

On multivariable logistic regression analysis, the following factors remained significantly associated with the decision to perform SLNB: age, type of breast surgery, detection by screening, method of diagnosis and tumor size assessed clinically.

Women having mastectomy, a screening-detected tumor, a preoperative diagnosis based on cytology and a palpable tumor, were significantly more likely of having SLNB whereas women aged 71 years and above were significantly less likely of having undergone SLNB. Predictors of SLNB performed in preoperatively diagnosed DCIS are presented in Table 5.

5.2 PAPER II

The 37 patients in the final analysis were examined between September 2011 and January 2015. The first eleven prospectively evaluated patients were excluded for technical reasons. CT imaging was performed with 5 mm slice thickness which caused too much image noise and in almost half of the patients, radioactive lymph nodes could not be visualized in at least one procedure. The following 37 patients were instead examined with 0.75 mm slice thickness and with attenuation correction which improved the image quality markedly. A flow chart over the study subjects is presented in Figure 7.

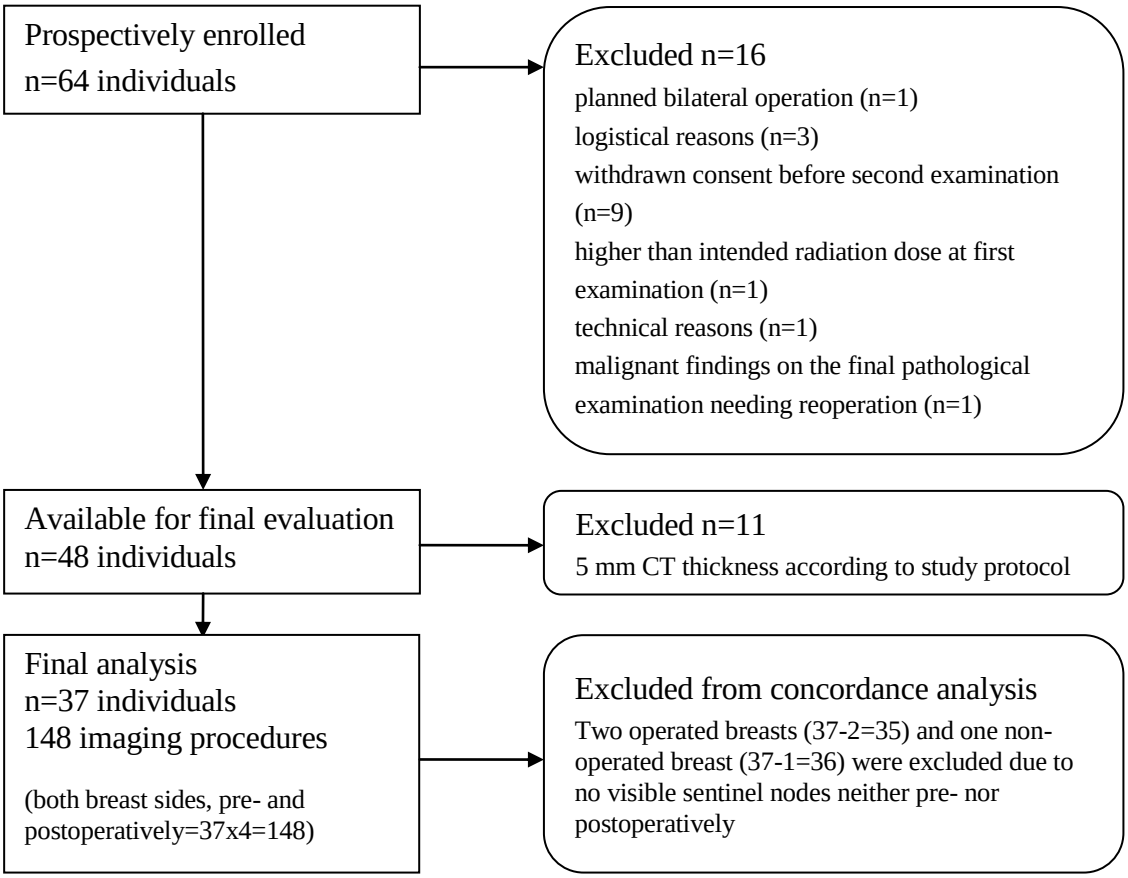

Figure 7 *Flow chart of study subjects in paper II.*

The median age of the 37 patients was 56 years (range 16-73), the median excised breast volume was 36.5 cm^3 (range 3-330) and the median weight of excised breast tissue was 22 g (range 1.5-172). This information was, however, missing in nine individuals. A SLN was visualized in 93.2% (138/148) of all procedures overall and in 91.9% (34/37) of postoperative procedures on operated sides to be compared with 93.7% (104/111; p=0.771) of all procedures on non-operated sides, including preoperative procedures on the operated side. Total or partial concordance was observed in 85.7% (30/35) on operated and 88.9% (32/36; p=0.735) on non-operated sides. None of the studied clinical and pathological characteristics differed significantly between discordant and concordant operated breast sides. Clinical and pathological characteristics on operated sides of the 35 evaluable patients are displayed in Table 6.

Table 6 Clinical and pathological characteristics according to SLN concordance analysis in operated breast sides.

	Partial or total concordance (n=30)	Discordance (n=5)	P
Age (years)			
Median	50	61	
Range	16-72	39-73	0.697[a]
BMI class			
Normal weight	21(70.0)	3(60.0)	
Overweight	6(20.0)	1(20.0)	
Obese	3(10.0)	1(20.0)	0.804[b]
Radiological lesion size (mm)			
Median	12	26	
Range	5-55	5-40	0.491[a]
Length of skin incision (mm)			
Median	40	52	
Range	15-55	25-60	0.142[c]
Distance from nipple (mm)			
Median	33	40	
Range	0-100	0-68	0.778[c]
Operated quadrant			
Upper outer	11(36.7)	1(20.0)	
Upper inner	3(10.0)	3(60.0)	
Lower outer	9(30.0)	1(20.0)	
Lower inner	6(20.0)	0(0.0)	
Central	1(3.3)	0(0.0)	0.095[b]
Weight of excised tissue (g)			
Median	23	14.3	
Range	2.7-172	1.5-69	0.727[a]
Excised breast volume (cm^3)			
Median	36.5	49.5	
Range	3.5-330	3-180	1.000[a]

Values in parentheses are percentages unless indicated otherwise.
P< 0.05 was considered statistically significant.
[a]Non-parametric continuous data= Mann-Whitney U test
[b]Non-parametric categorical data= Pearsons Chi-square test
[c] Parametric continuous data= Independent samples t-test (equal variance)
SLN sentinel lymph node, BMI Body Mass Index.

5.3 PAPER III

Of 264 eligible patients, 40 withdrew or were excluded for different reasons, leaving 224 patients from 13 recruiting hospitals in the final analysis. A CONSORT diagram over both trial is displayed in Figure 8.

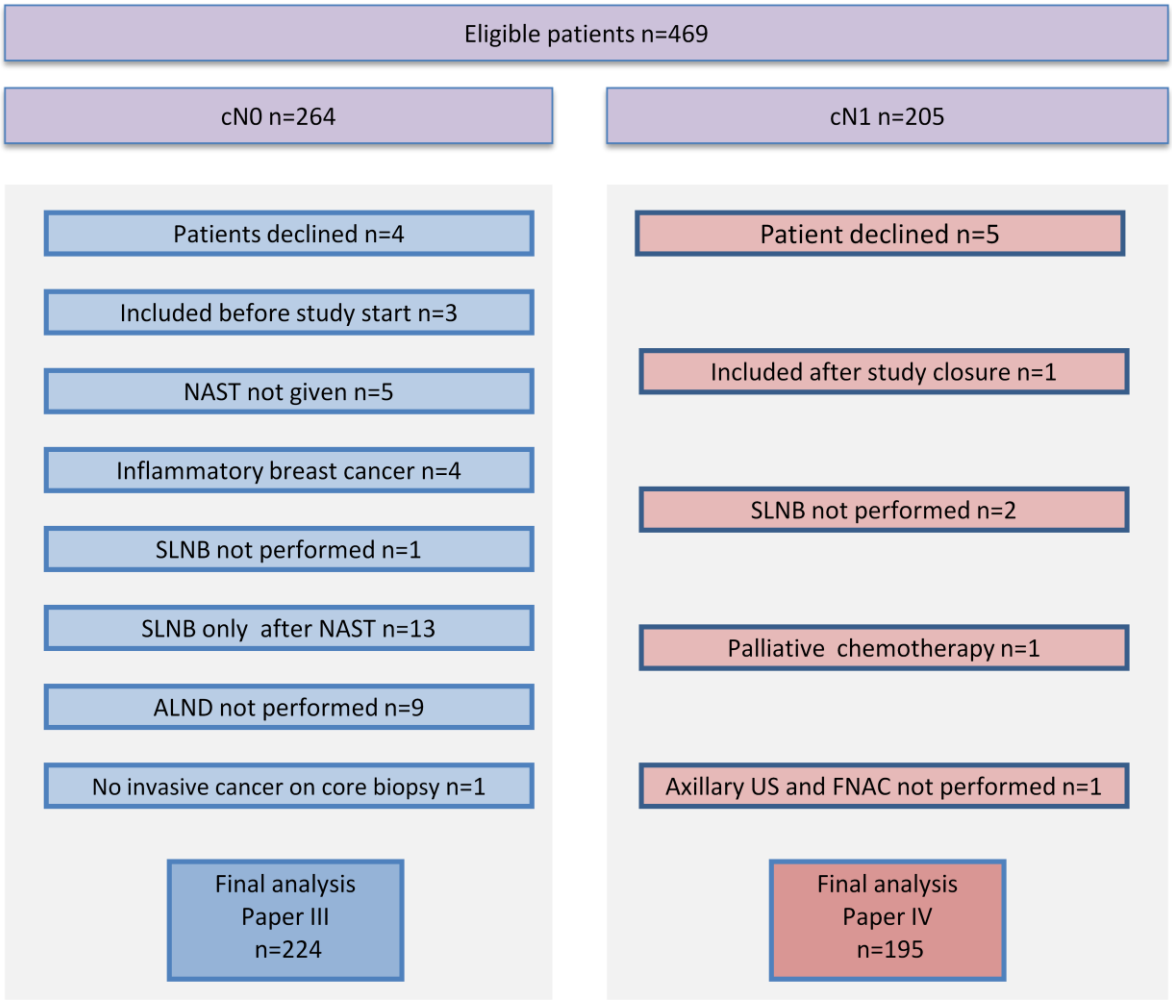

Figure 8 *CONSORT diagram over the two trial arms in paper III (blue) and IV (red). cN0 clinically node-negative, cN1 clinically node-positive,NAST neaodjuvant systemic therapy, SLNB sentinel lymph node biopsy, ALND axillary lymph node dissection, US ultrasound, FNAC fine needle aspiration cytology.*

The proportion of patients recruited from each site is displayed in Figure 9. Median age in paper III was 47 years (range 22-78) and median radiological tumor size at diagnosis was 39 mm (range 9-127). For clinicopathologic and treatment characteristics for both trial arms, see Table 7.

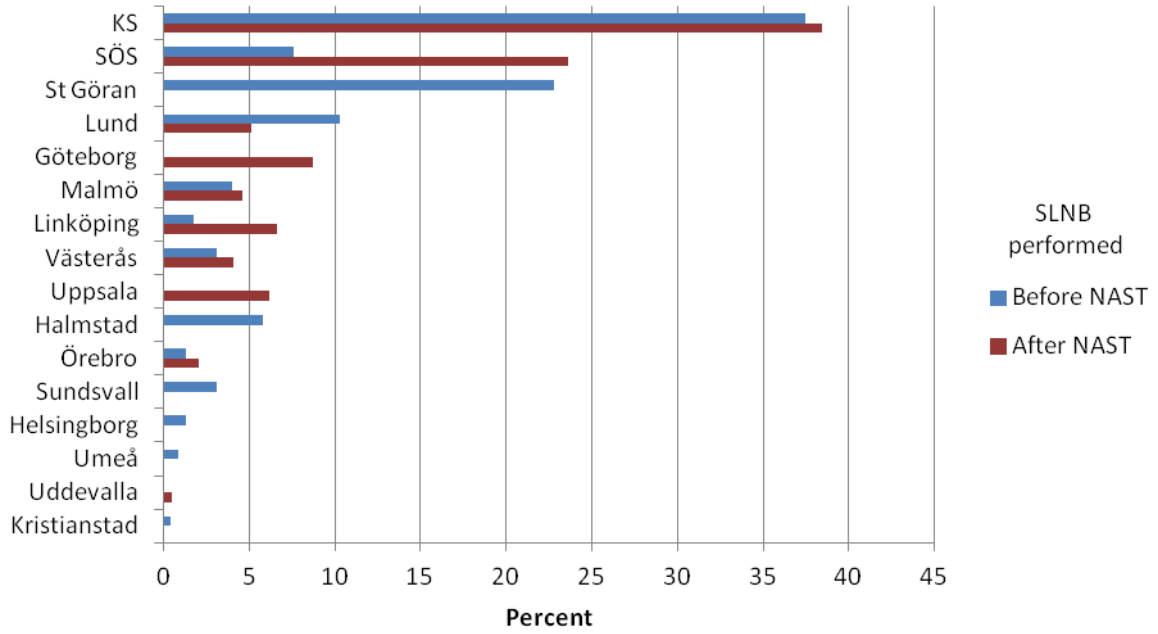

Figure 9 *Recruiting hospitals for papers III (blue) and IV (red) respectively.*

Lymphatic mapping was performed with radiolabeled colloid and Patent Blue V dye (dual mapping) in 95.5% (213/223) of patients. At least one SLN was identified in all patients before NAST leading to an IR of 100% (224/224). The median number of retrived SLNs was two (range 1-11). Half of all patients had a positive SLNB (112/224). The median number of macrometastases was 1 (range 1-6). Almost 77% (86/112) of patients with a positive SLNB had only negative lymph nodes after NAST, including two patients with ITC, ypN0(i+). Nine patients had a negative SLNB before NAST but at least one positive lymph node after NAST, resulting in a FNR of 7.4% (95% CI 4.0-13.5) and an accuracy of 96.0% (215/224). For crosstabulation of SLNB results before NAST and corresponding axillary lymph nodes after NAST, see Table 8. The maximum number of positive lymph nodes was two. There was no significant difference between patients with a false-negative compared with a true-positive or true-negative SLNB regarding the factors listed in Table 7. The proportion of patients with best clinical or radiological tumor response was 22.2% (2/9) and the best pathologic tumor response 11.1% (1/9) among patients with a false-negative SLNB. The corresponding figures for patients with a true-positive or true-negative SLNB upfront were 42.3% (91/215, p=0.089) and 30.7% (66/215, p=0.036) respectively.

Repeat SLNB after NAST was attempted in 98 patients and dual mapping performed in 86.7% (85/98). In 68 (69.4%) patients at least one SLN was identified. The median number of repeat SLNs was 1 (range 1-5). The FNR for repeat SLNB was 25.0% (3/12).

Table 7 Clinicopathologic and treatment characteristics of the whole trial population.

	paper III (%)	paper IV (%)
No. of patients	224	195
Median age, years	47, range 22-78	50, range 27-84
Radiological T stage		
T1	18 (8.0)	25 (12.8)
T2	149 (66.5)	94 (48.2)
T3	57 (25.4)	61 (31.3)
T4d (inflammatory)	0 (0.0)	15 (7.7)
Histological type		
Ductal	181 (81.5)	158 (83.6)
Lobular	28 (12.6)	14 (7.4)
Other	13 (5.9)	17 (9.0)
Unknown	2 (0.9)	6 (3.1)
Nottingham histological grade		
I	5 (3.1)	1 (0.7)
II	76 (46.9)	79 (55.6)
III	81 (50.0)	62 (43.7)
Unknown	62 (27.7)	53 (27.2)
ER positive	137 (61.2)	134 (68.7)
PR positive	102 (45.5)	95 (48.7)
HER2 positive	72 (32.3)	62 (31.8)
Unknown	1 (0.4)	
Neoadjuvant therapy		
Anthracycline plus taxane	199 (88.8)	184 (94.4)
Anthracycline only	10 (4.5)	7 (3.6)
Other type	13 (5.8)	3 (1.5)
Aromatase inhibitor	2 (0.9)	1 (0.5)
Anti-HER2 therapy	68(94.4)	58 (93.5)
Breast-conserving surgery	65 (29.0)	51 (26.2)

ER estrogen receptor, PR progesterone receptor, HER2 human epidermal growth factor receptor 2

Table 8 Crosstabulation of SLN status before NAST and overall axillary nodal status.

	Overall axillary nodal status[a]		
SLNB before NAST	**Positive**	**Negative**	**Total**
Positive	112	0	112
Negative	9	103	112
Total	121	103	224

[a]Lymph node status in SLNs before, SLNs after NAST if performed, and non-SLNs after NAST.

Sensitivity 92.6% (112/121), specificity 100.0% (103/103) and accuracy 96.0 % (215/224).

SLN sentinel lymph node, SLNB sentinel lymph node biopsy, NAST neoadjuvant systemic therapy

5.4 PAPER IV

Of 205 eligible clinically node-positive patients, 195 patients from ten hospitals were in the final analysis. A flow chart for both trial arms is presented in Figure 5. All patients had cytologically confirmed node-positive disease at diagnosis. The median age was 50 years (range 27-84) and the median radiological tumor size was 40 mm (range 11-60). Fifteen patients had inflammatory breast cancer (IBC). Clinicopathological and treatment characteristics are presented in Table 7. Lymphatic mapping was performed with radiolabeled nanocolloid and Patent Blue V dye in 87.5% (168/192) of patients. In 3.6% (7/192) magnetic tracer alone or in combination with blue dye was used. Overall IR was 77.9% (152/195) and with dual mapping regardless of method, IR improved to 80.7% (138/171). After excluding patients with IBC, IR improved slightly to 79.4% (143/180) and with dual mapping IR improved somewhat further to 82.8% (130/157). The median number of retrieved SLNs was two (range 1-5). SLNB was positive after NAST in 52.0% (79/152) and at least one macrometastasis was found in 88.6% (70/79) of patients, with a median of one (range 1-4). The median number of non-SLNs was 11 (range 3-41) and 52 out of 79 (65.8%) patients with a positive SLNB had additional positive non-SLNs. Of all 195 patients, 124 (63.6%) had residual nodal disease after NAST.

SLNB was negative after NAST in 73 (48%) of 152 patients. Thirteen of them had positive non-SLNs for an overall FNR of 14.1% (13/92). A comparison of lymph node status in SLNs and non-SLNs after NAST is presented in Table 9.

Table 9 Comparison of lymph node status in SLNs and overall axillary lymph node status after NAST.

	Overall axillary nodal status (SLNB and ALND)		
SLNB	**Positive**	**Negative**	**Total**
Positive	79	0	79
Negative	13	60	73
Total	92	60	152

Sensitivity 85.9% (79/92), specificity 100.0% (60/60), positive predictive value 100.0% (79/79), negative predictive value 82.2% (60/73).
SLN sentinel lymph node, SLNB sentinel lymph node biopsy, NAST neaodjuvant systemic therapy, ALND axillary lymph node dissection.

Among the false-negative patients the median number of positive lymph nodes was one (range 1-9) and the median number of axillary lymph nodes including SLNs was 12 (range 5-20). Among the false-negative cases there were two patients with IBC and three patients with isolated tumor cells (ITC) in their SLNs. FNR calculated for different clinical scenarios are presented in Table 10.

There was no difference between patients with a false-negative compared with a true-positive or true-negative SLNB in relation to the factors displayed in Table 7 or in clinical or radiological response to NAST in neither tumor nor lymph nodes. However, there were significantly fewer patients with a complete or near complete (Sataloff A) pathologic response in the tumor (7.7%) and lymph nodes (0%) in the false-negative group. This was compared with the true-positive or true-negative group in which the corresponding figures were (35.3%, p=0.044) and (27.3%, P=0.01) respectively.

Table 10 False-negative SLN rates calculated for different clinical scenarios.

Scenario	True-pos (n)	False-neg (n)	FNR[a] (%)
Overall	79	13	14.1
Dual mapping performed	71	11	13.4
IBC excluded (n=15)	76	11	12.6
ITC considered ypN+	87	10	10.3
SLNB with 1 node retrieved	31	11	26.2
SLNB with ≥ 2 nodes	48	2	4.0
SLNB with ≥ 3 nodes	23	0	0.0

[a]Calculated as the number of patients with a false-negative SLN in each scenario, divided by the number of false-negative and true-positive SLNs in the same scenario.
NAST neoadjuvant systemic therapy, FNR false negative rate, SLN sentinel lymph node, SLNB sentinel lymph node biopsy, IBC inflammatory breast cancer, ITC isolated tumor cells, SLN sentinel lymph node, SLNB sentinel lymph node biopsy, FNR false negative rate.

6 DISCUSSION

6.1 PAPER I

The incidence of DCIS has increased since the introduction of mammography screening and accounts for about 10% of all newly diagnosed breast cancers[9]. DCIS is a pre-invasive condition where cancer cells grow in the mammary ducts but respect the basal membrane and thus are not able to metastasize. However, SLN metastasis is found in 5-13% [130] of preoperatively and 0-4% [173, 174] in postoperatively diagnosed pure DCIS. The discrepancy is due to upgrading to microinvasive or invasive breast cancer on definitive pathology reports [174, 175]. The rationale for SLNB in larger DCIS is to compensate for sampling limitations, so that adjuvant treatment decisions are based on correct TNM staging. According to the Swedish National Guidelines valid in 2007, when this study was planned, SLNB should be considered in case of a clincial or radiological tumor extent larger than 2 cm and high nuclear grade on preoperative core biopsy or high grade atypia on FNAC.

The primary aim with this study was to investigate the national incidence of SLN metastasis in pure DCIS based on the final pathology report. The secondary aim was to examine whether a more thorough examination of the tumor blocks could substitute for SLNB. Our hypothesis was that SLNB was performed too liberally and also in cases that did not fulfill the National Guidelines criteria. Interestingly, according to the registered data for patients having BCS, SLNB was performed in three out of four cases despite the criteria from the guidelines not being fulfilled. During the same period, the corresponding figure for patients having a mastectomy was one out of two cases. In our study, only 0.7% of patients with a postoperative diagnosis of pure DCIS and combined with the performance of a SLNB developed SLN metastasis. None of the SLN-positive patients had more than one metastasis and there were no patients with non-SLN metastases. A Dutch review of 21 studies reported an average incidence of 4% for SLN metastasis in postoperatively diagnosed pure DCIS [175]. However, the range was wide (0-18%), possibly representing differences in tissue sampling techniques and the proportion of large samples. The more extensively the tumor blocks are examined, the greater the chance that occult invasion may be revealed. This is supported by the results from our study in which occult invasion indeed was discovered after re-evaluation and additional sectioning of the tumor blocks, but was found to the same extent in both SLN-negative and SLN-positive patients. However, the procedure is labour-intensive and costly and therefore not suitable for most clinical settings.

We did not find any recognized predictive factor for SLN metastasis that was signficantly representative upon regression analysis in our study. This may be due to the small number of events involved. However, this in line with the results found by Cox et al and Tan et al [176, 177]. On the other hand, two out of five patients with SLN metastasis did not fulfil the criteria stated in the Swedish National Guidelines valid at the time of surgery.

On multivariable logistic regression analysis, total mastectomy, screening-detected tumor, preoperative diagnosis made by cytology and palpable tumor were all predictors for choosing to perform SLNB. SLNB is difficult to perform after mastectomy, invasiveness is hard to rule out with cytology and palpability raises suspicion of invasiveness. These are all logical predictors for choosing to perform SLNB. The reason for screening-detected tumor being a predictor is more intriguing. It is unusual with clincially-detected DCIS. Those proportionally few cases in our data may have been an incidental postoperative finding where preoperative suspicion was not raised and thus SLNB not considered. Older women were instead less likely to be offered SLNB. Instead primary ALND was performed in 8.7% (10/115) of older women with available data compared with 1.7% (8/465) in postmenopausal and 0.5% (1/191) in premenopausal women.

Our results, which were based on validated national data from 55 reporting hospitals with an almost 100% coverage in the reporting of new breast cancer cases, found a markedly low incidence of SLN metastasis in postoperatively diagnosed pure DCIS. However, some critics claim that this information is of little use in preoperative decision-making on whether to perform SLNB or not if BCS is planned. On the contrary, since the incidence of SLN metastasis was so low, our recommendation, in line with most international guidelines, is to postpone nodal staging until the final pathology report is available unless a mastectomy is planned.

6.2 PAPER II

After triple assessment almost 10% of patients are planned to receive a diagnostic breast excision in order to achieve a conclusive diagnosis [9]. Those patients with an invasive form of breast cancer, diagnosed after excisional surgery, are more often node-negative compared with patients with a conclusive cancer diagnosis prior to surgery. Since SLNB is associated with morbidity, although to a lesser extent than ALND [82, 94], SLNB should only be performed where there is a clear indication. Therefore, SLNB as a second operation for the purpose of nodal staging is preferable. However, earlier results with a secondary SLNB after prior diagnostic excisional surgery has been associated with lower IR [133, 178] and higher FNR especially in large excisional biopsies [101, 132]. Borgstein et al and Feldman et al both used radioactive tracer without blue dye and injected the radioactive tracer into the breast tissue surrounding the surgical cavity. Based on these results, SLNB was considered inaccurate after prior diagnostic surgery explained by lymph vessel disruption and postoperative inflammation, altering lymphatic drainage and redirecting lymph flow so that the mapped SLN would not reflect the “true SLN” [133]. In 2007, Celebioglu et al published a prospective multicenter study evaluating the safety and accuracy of SLNB after prior diagnostic excisional surgery. Dual mapping was applied and the observed IR was 96% and the FNR 10% for this group. Although the follow-up was short, the authors concluded that SLNB was safe in this setting [137]. This was in line with the results reported by Wong et al in 2002, who did not find any statistical difference in IR or FNR compared with patients having a needle biopsy performed prior to definitive surgery. The same group also observed

that IR was significantly improved if the radioactive tracer was injected dermally instead of peritumorally [135]. This observation agrees with the results by McMaster and colleagues [179].

Contrary to these successful results, and the main reason why we decided to perform our study, were the findings reported by Estourgie et al, who observed a discrepancy in lymph drainage patterns in 17 out of 25 study patients and a discordance in 7 out of 25 (28.0%) patients examined with planar lymphoscintigraphy before and after prior excisional surgery [180].
We decided to use hybrid SPECT/CT imaging instead of planar lymphoscintigraphy for the comparative imaging studies. With SPECT, three-dimensional, instead of two-dimensional, scintigraphic functional images are produced which in combination with precise anatomical information from CT, facilitates the comparison and localization of radioactive SLNs [181]. The second SPECT/CT procedure was performed approximately six weeks postoperatively. This interval was mainly chosen because reoperations for nodal staging often take place after six weeks from the initial surgical procedure. Additionally, an interval of more than 36 days between the surgical interventions improved detection rates in a recent study by Renaudeau et al [182]. This is also supported by high SLN identification rates years after earlier aesthetic breast surgery [183, 184].

For practical reasons we used standardized superficial periareolar injections of the radioactive tracer positioned at six o´clock both pre- and postoperatively. The contralateral side was used as a control for evaluation of reproducibility with repeat SPECT/CT imaging. To the best of our knowledge, repeat SPECT/CT imaging for evaluation of lymph drainage alterations after prior diagnostic surgery has not been reported before. Asadi et al used planar lymphoscintigraphy to evaluate lymph drainage changes in 18 patients. A comparison was performed of lymphatic mapping preoperatively on the day of surgery with the day after an excisional biopsy using intra-dermal periareolar injections of radioactive tracer. Apart from two patients with non-visualization, the SLNs were in the same location postoperatively [185]. Noushi et al later conducted a comparison study of lymph drainage patterns in 39 patients evaluated with SPECT/CT imaging after sequential subareolar and peritumoral injections of radiocolloid with intervals of 2-7 days, without any surgery performed between studies. High rates of discordance were found in lymph node mapping to the internal mammary and axillary lymph nodes. The conclusion reached was that the location and depth of radioactive tracer injection may have implications for both nodal staging and patient outcome [186]. In our study there was no statistical difference in concordance (partial or total) between operated and non-operated sides, even though concordance was lower on operated sides. Similarly, the visualization of SLNs was not significantly different for procedures performed postoperatively on operated sides in comparison with procedures on non-operated sides or preoperatively on operated sides. Excision volume had no significant impact on discordance rates on operated sides. This is in line with the observations made by Haigh et al who found that the excision volume did not affect the IR of SLNs [187]. We found significantly higher BMI and higher age in procedures with non-visualization, which

corroborate with earlier results by Derossis et al who found that BMI was significantly higher among patients with failed lymphatic mapping [188]. Lerman et al observed that SPECT/CT imaging was superior to planar lymphoscintigraphy in visualizing SLNs in overweight patients [189]. Despite only 37 evaluated patients, our results support performing SLNB after previous excisional surgery with only a tendency for higher discordance rates on operated sides. Larger studies are however welcome to further validate our data. The SLNB technique appears to have a margin for error given low recurrence rates [115] and equivalent survival [117] after long time follow-up, which is most likely compensated for by modern adjuvant therapies.

6.3 PAPER III & IV

SLNB is considered gold standard nodal staging procedure in early-stage breast cancer and ALND can be safely omitted in case of a negative SLNB [117]. NAST has traditionally been offered to patients with locally advanced or inflammatory inoperable stages of the disease and ALND is then the traditional nodal staging procedure followed by locoregional radiotherapy. However, in the last three decades, the indications for NAST have expanded to also encompass patients with operable breast cancer with aggressive tumor biology, rendering them candidates for adjuvant chemotherapy [145]. Survival rates are comparable with this sequence, and in addition NAST can downsize tumors so that more patients can be candidates for BCS after NAST. Chemosensitivity can be evaluated in vivo and treatment adjusted if required [146]. Since the proportion of patients with node-positive disease is lower in operable breast cancer, and NAST has the potential for nodal conversion in 40-70% of patients, SLNB has also been evaluated worldwide in the neoadjuvant setting [155, 190]. SLNB before NAST in clinically node-negative patients with breast cancer has been evaluated in a number of small single-institution studies. The reported IR is excellent and FNR 0% if a complementary ALND was performed in SLNB-negative patients [191-193]. However, most studies omitted ALND if SLNB was negative and reported absence of recurrences after a median 10-36 months of follow-up [140, 194, 195].

To the best of our knowledge, our trial is by far the largest in which an ALND was performed, irrespective of the result of the SLNB upfront and in addition within a multicenter setting. Our patients were recruited from 16 different Swedish hospitals, mostly large-volume and university-affiliated. There are still large regional differences in the proportion of patients being offered NAST, especially in operable stages of the disease, even though the differences are decreasing. Since current guidelines from the National Board of Health and Welfare (Socialstyrelsen) recommend NAST to patients with operable aggressive breast cancer stage II, the differences will probably decrease further [196]. The IR for SLNB before NAST was in paper III 100%, including three patients in whom SLNB was identified only due to a suspicious finding upon digital exploration. This high IR agrees with earlier studies performing SLNB as part of primary surgery in clinically node-negative patients [101, 197]. Dual mapping was performed on 95.5% of patients in our trial. This is known to improve detection rates in early-stage breast cancer, especially in a multi-institutional practice [96]. In

paper IV, when SLNB was performed after NAST, dual mapping also improved the IR, which is in line with the results from both the SENTINA study and the ACOSOG Z1071 trial [198, 199]. However, corresponding with the results in the SENTINA study, repeat SLNB was associated with low IRs, despite dual mapping in most cases. The reasons for this are probably post-surgical tissue scarring and post-NAST inflammatory debris in the lymph vessels altering lymphatic drainage [198]. In clinically node-negative patients, SLNB performed after NAST is associated with both lower IR and higher FNR compared with SLNB performed upfront [141, 200].

The number of patients with a negative SLNB upfront and at least one positive axillary lymph node after NAST was nine for a FNR of 7.4 % (95% CI 4.0-13.5). We did not find any significant difference in clinical or treatment characteristics between patients with a false-negative compared with a true-positive or true-negative SLNB. We do not know if those nine "false-negative" patients had additional overlooked positive lymph nodes during primary surgery or if they represent metastases that developed during NAST.

Clinical response to NAST did not differ significantly between patients with a false-negative compared with a true-positive and true-negative SLNB, neither for the arm with SLNB performed before or after NAST. However, a pathologically complete or near complete response in the breast was significantly less frequent among patients with a false-negative compared with a true-positive and a true-negative SLNB in both the trial arms. One of the patients with a false-negative SLNB before NAST progressed clinically during NAST. Pathological response evaluation was graded according to the definitions stated by Sataloff et al [169]. Sataloff observed a poor correlation between clinical and pathological response, which is in line with our own observations. He further concluded that patients whose tumors had the best pathologic response also had the best outcome. Pathological complete response (pCR) is an excellent predictor of outcome and the CTNeoBC pooled analysis by Cortazar et al shows that pCR correlates with improved survival and that the correlation is strongest for patients with aggressive tumor subtypes [148]. The pCR definition recommended by The Breast International Group-North American Breast Cancer Group (BIG-NABCG) collaboration is no residual invasive carcinoma in the breast and all sampled lymph nodes [171]. The latter is important since residual nodal disease impacts negatively on outcome even if there is no residual disease in the breast [201, 202]. The BIG-NABCG recommends using residual cancer burden (RCB) for the assessment of pathological residual disease [171]. RCB is a continuous variable with numerical cut points to define four classes based on prospectively sampled data, including: tumor size, cellularity and number and size of the largest metastasis. Since the response evaluation in our trial is based on retrospectively collected data from pathology reports, we chose to classify pathological response according to definitions by Sataloff et al.

In clinically node-negative patients, SLNB can be performed both before and after NAST according to international guidelines [116, 203]. Proponents of performing SLNB after NAST argue that only one surgical intervention is needed and that more patients can take

advantage of nodal downstaging secondary to NAST and hence be spared ALND if SLNB is negative. However, with this sequence, there is uncertainty on upfront nodal stage. Ultrasound followed by FNAC has in some studies a sensitivity of only 20-25% in finding nodal metastases [75, 76]. If nodal metastases are overlooked upfront and SLNB is false-negative after NAST in approximately 11% [141, 204] of cases, there is a clear risk of locoregional undertreatment. There is a lack of evidence on how to treat the axilla after both a negative and a positive SLNB post NAST. Therefore it seems safer to recommend SLNB before NAST in clinically node-negative patients at diagnosis pending results from the randomized NSABP B-51 and Alliance A011202 trials investigating locoregional treatment after NAST [205, 206].

In paper IV, clinically node-positive patients with cytologically-verified nodal disease were eligible. The IR was, as expected, lower compared with earlier results reporting SLNB after NAST in clinically node-negative patients, but improved somewhat with dual mapping and slightly further after excluding patients with IBC. These results are in line with the IR of 80.1% in arm C of the four- armed SENTINA study [198]. A low IR in patients with biopsy-proven nodal disease before NAST is acceptable since the alternative would have been ALND. We included IBC in paper IV despite ASCO guidelines recommending against the performance of SLNBs in IBC, also after a positive clinical response to NAST [116]. We only found two small studies specifically evaluating IBC in the neoadjuvant setting and both authors concluded that SLNB was unsuitable [207], but one of them did not use the dual mapping technique [144]. Our results regarding IR and FNR, improved when the cohort of IBC patients were excluded, but only marginally and further studies are warranted.

In clinically node-positive patients, the overall FNR associated with SLNB after NAST is unacceptably high [142]. However, if two or more SLNs are retrieved, the FNR decreases, in our study to 4%, which agrees with earlier studies [208]. A limitation of our trial was that axillary reevaluation after NAST was not performed. The SENTINA study arm C [209] and the ACOSOG Z1071 trial both enrolled clinically node-positive patients and reevaluated the axilla with ultrasound (AUS) after NAST. In the ACOSOG Z1071trial, fewer patients with sonographically normal-looking lymph nodes were node-positive at surgery and the FNR based on the AUS findings was lower, although not significantly different [210]. Even though the accuracy for AUS in predicting nodal status after NAST is too low, it can be used to stratify patients suitable for SLNB with normal-looking lymph nodes and, therefore lower risk of false-negative SLNs. Another potential way of lowering the FNR, is to expand the definition of a positive node to also encompass ITC. In our trial (paper IV), the FNR decreased to 10.3% after having excluded three patients with ITC from the false-negative cases. This is in line with Boileau et al in the SN FNAC trial, where the FNR decreased from 13.3 to 8.4% [211]. In our study, IHC was not mandatory as it was in the SN FNAC study. Even minimal residual nodal disease after NAST is thought to be of clinical relevance in the neoadjuvant setting, unlike the situation when primary surgery is performed [212]. The 7th edition of the AJCC staging manual recommends including the size of the fibrous stromal reaction around the tumor deposit, which in some cases might upgrade ITCs to node-positive

disease. Finally, marking the cytologically confirmed nodal metastasis prior to NAST with a clip or a radioactive seed and removing that node at definitive surgery together with SLNB, also has the potential to lower the FNR as shown in a prospective study by Caudle et al [213].

6.4 METHODOLOGICAL CONSIDERATIONS

To be able to draw conclusions regarding a population based on observations from a sample, it is necessary to assess if the conclusions are correct for the people in the study (internal validity) and for other groups outside the sample (external validity).

The internal validity is affected by two types of errors: Random errors and systematic errors.

Random errors cause unprecise results that differ when you repeat your measurements. Random errors can be minimized with a larger study size. In paper III, an a priori power analysis was conducted to calculate a sufficient sample size at a power of 80% but with a relatively wide CI around the point estimate (FNR), which was accepted since all patients was subject to NAST.

Systematic errors are reproducible inaccuraccies that deviate consistently in the same direction causing a lower validity. Selection bias, information bias and confounders are different types of common systematic errors.

Selections bias: In paper II, the two imaging procedures required a couple of hours time each, hence employed women were less prone to participate compared with unemployed or retired women. Further we chose patients with probably benign tumors as a model for our study to avoid reoperation for nodal staging, but we have assumed that there is no major difference in lymph drainage pattern compared with a diagnostic excisional biopsy for a postoperatively diagnosed breast cancer. In paper III clinically node-negative patients were eligible. These women were in most cases enrolled from academic high-volume centres, in which the oncologists seemed to be more prone to plan for NAST in operable stages of the disease compared with low-volume centres.

Information bias: In order to avoid information bias in paper I, the two pathologists had no information which slide belonged to a patients with tumor deposits in the SLNs or not when assessing microinvasion. In paper II the reviwers did not know which breast was the operated one when examining the hybrid SPECT/CT images. However, in some cases it was possible to decide from the images. In paper III and IV, response was assessed retrospectively with information extracted from medical records and pathology reports and thus misclassification is possible. In paper IV SLNB was attempted after NAST in conjunction with the mandatory ALND, hence there is a possibility that the SLNs were retrieved ex vivo among the harvested axillry nodes to save time and thus the IR in reality may be even lower.

Confounding: In paper I, we choose controls for each case matched for age, tumor extent and grade to avoid mixture of effects or confounding. There could still however be additional unknown confounders.

Type II error: In paper I, we did not find any significant risk factors for SLN metastasis which could be due to low case numbers. In paper II, the study size was small and with null findings there is a risk for incorrectly having accepted the null hypothesis.

In paper I the external validity is high since the study is based on national data from the Swedish National Breast Cancer Registry, which has high validity and coverage.

In paper II the internal validity is high, but the results are based on only 37 patients and therefore further studies are needed to validate the results.

Paper III and IV are based on a large prospective national multicenter trial with both low-volume and high-volume hospitals. In paper III the accrual goal for the number of participants was reached and the results can thus be regarded as having a high internal as well as external validity.

7 CONCLUSIONS

1. The incidence of SLNB metastasis in postoperatively diagnosed pure DCIS is very low and we could not find any significant predictive risk factor for SLN metastasis.

2. In patients with preoperatively diagnosed pure DCIS, SLNB is only indicated if a mastectomy is planned or if findings highly suspicious of invasive disease are present.

3. In postoperatively diagnosed pure DCIS, additional tumor sectioning can reveal occult microinvasion. However, this finding appears to be equally common in patients whether tumor deposits in SLNs are encountered or not, reflecting limitations in tissue sampling and the standardized pathological examination of tumor specimens.

4. Prior diagnostic excisional breast biopsy alters the lymphatic drainage pattern from the breast to the axillary lymph nodes. However, the magnitude of change was not significantly different in operated compared with non-operated breast sides.

5. Our results support the feasibility of SLNB after prior diagnostic breast surgery.

6. SLNB performed before NAST in patients with clinically node-negative breast cancer is highly reliable. If SLNB is negative, ALND can be omitted provided good clinical tumor response to NAST.

7. SLNB after NAST in patients with clinically node-positive breast cancer is feasible, however the IR is lower than in clinically node-negative patients and the FNR unacceptably high if only one SLNB is retrieved.

8. Omission of ALND after NAST in clinically node-positive patients upfront, should only be considered if two or more SLNs are retrieved.

8 FUTURE PERSPECTIVES

This thesis has attempted to answer some of the questions surrounding nodal staging and specifically the indications and limitations of SLNB in different breast cancer scenarios that clinicians are facing today. The surgical therapies available to treat breast cancer have evolved from Halsted's extensive radical disfiguring mastectomy to the current considerably less damaging BCS, sometimes incorporating oncoplastic techniques to restore the shape and volume of the breast, together with SLNB in clinically node-negative patients. In combination with improvements in adjuvant therapy, the survival rates for breast cancer are now among the highest compared with other cancer types. Approximately 90% of breast cancer patients are alive five years after the initial diagnosis. However, since all surgical procedures have side-effects and associated morbidity, the indications must be clear.

In patients with preoperatively diagnosed DCIS planned for BCS, most current international guidelines recommend that SLNB should not be performed unless there is a high suspicion of invasive disease [116, 203]. However, SLNB is warranted if mastectomy is planned, as performing a secondary SLNB postoperatively is precluded, even though there are reports of this having been successfully performed in selected cases [214].

In addition, after prior excisional breast surgery including BCS, SLNB seems sufficiently accurate to be performed as a secondary surgical procedure, in cases where invasive cancer is encountered on the final pathology report [215].

In recurrent breast cancer, a repeat SLNB has been attempted and seems feasible according to a recent meta-analysis, especially if the original axillary surgery was SLNB. However, abberant lymphatic drainage patterns visualized on planar lymphoscintigraphy were significantly more common if the original axillary surgery had been an ALND [216]. In recurrent breast cancer, planar lymphoscintigraphy should probably be routine as aberrant drainage pathways and extra-axillary tumor-positive SLNs can be revealed, that may change both surgical and adjuvant therapy decisions including radiotherapy plans [217]. Whether SPECT/CT imaging can be of additional value has been adressed in a recent study [218]. However, this can be studied further, including the correlation between time to recurrence and its impact on lymphatic regeneration and detection rates.

In early-stage breast cancer, there is much evidence to support that ALND can be safely omitted in case of a negative SLNB without a negative impact on either survival or recurrence rates [115, 117].

The issue is instead how to treat patients with a low tumor burden in the SLNs given current adjuvant therapy options. According to international guidelines, ALND can be omitted after BCS in clinically node-negative patients if no more than two positive SLNs are present and raditohterapy planned [116]. A Swedish-based international randomized trial called

SENOMAC (www.senomac.se) with the primary endpoint DFS, is currently enrolling patients with maximum two positive SLNs, irrespective of type of breast surgery being planned.

The issue of whether to replace ALND with axillary radiotherapy in case of a positive SLN has also been investigated. In the randomized multicenter non-inferiority AMAROS trial, both survival and the proportion of axillary recurrences were comparable between groups after a median of six years of follow-up but the patients having axillary radiotherapy had significantly less arm morbidity in comparison with the ALND group. However, the trial was underpowered with very few events [219].

We have shown that SLNB performed before NAST in clinically node-negative patients is highly reliable and complementary ALND can be omitted after NAST in case of good clinical response. It is however important to carefully monitor patients in whom ALND is omitted with attention to regional recurrences in the future. Patients with a positive SLNB upfront can be enrolled into the SENOMAC trial and thus randomized to receive a complementary ALND or not, and followed according to the trial protocol.

SLNB can be attempted on patients who are clinically node-positive at presentation if clinically downstaged in the axilla after NAST, according to evaluation with AUS in addition to physical examination. However, if only one negative SLNB is mapped a complementary ALND should be performed. If two or more negative SLNs are retrieved, ALND may be omitted. The question of how best to treat the axilla after NAST is much debated and evidence lacking. This issue is under investigation and results pending from two important randomized controlled trials, the The NSABP B-51/RTOG 1304 trial evaluating the benefit of regional nodal radiotherapy in biopsy-proven node-positive patients with a nodal pCR after NAST according to SLNB or ALND and the Alliance A011202 trial, which enrolls patients with residual positive lymph nodes after NAST onto ALND with nodal radiotherapy, or no ALND but instead nodal and axillary radiotherapy [206].

In our multicenter trial, the amount of fibrosis in the residual tumor bed and lymph nodes after NAST was assessed retrospectively with information from the pathology reports. We did not find any significant difference in the amount of fibrosis between false-negative compared with true-positive or true-negative SLNs. However, this information was unfortunately often lacking or difficult to interpret. It would therefore be interesting to let a senoir pathologist centrally reexamine the tissue slides from the tumors and nodes and correlate the findings with the outcomes of SLNB. It would also be interesting to sequence the genome from the patients' tumor tissue blocks in order to correlate gene expression patterns and molecular subtypes with response to NAST and false-negative rates.

9 SAMMANFATTNING (SWEDISH SUMMARY)

Bröstcancer är kvinnans vanligaste cancerform och varje år insjuknar i Sverige cirka 8000 kvinnor och 60 män i bröstcancer. Lymfkörtelstatus, det vill säga om bröstcancern har spridit sig till de närbelägna lymfkörtelstationerna eller inte, och omfattningen på denna spridnig är den viktigaste prognosfaktorn vid bröstcancer. Lymfkörtelstatus har tidigare undersökts med lymfkörtelutrymning som innebär att ett drygt tiotal lymfkörtlar opereras bort från armhålan dit tumörceller från en bröstcancer oftast sprider sig först.

På 1990-talet kom portvaktskörtelkirurgi (sentinel node-biopsi) att allt mer ersätta lymfkörtelutrymning för stadieindelning. Portvaktskörtelkirurgi innebär att endast den första, eller de första, dränerande lymfkörtlarna från bröstet opereras bort. Är den eller dessa lymfkörtlar friska, så är de övriga lymfkörtlarna också friska med stor säkerhet och patienten slipper lymfkörtelutrymning. Portvaktskörtlarna hittas genom att man sprutar ett radioaktivt spårämne i bröstet innan operationen, vanligtvis tillsammans med ett blått färgämne som sprutas i bröstet när patienten är sövd. Dessa båda ämnen tas upp och sprider sig i lymfbanorna och ansamlas i den eller de första lymfkörtlarna som mottar lymfvätska från bröstet. Vid operationen används en liten handhållen gammakamera som riktas mot armhålan och som känner av radioaktiviteten i portvaktskörteln och ger ifrån sig ett ljud när man är i närheten. Hudsnittet läggs där man får som högst utslag med gamma proben. Vid dissektionen följer kirurgen i tillägg till ljudet även de blåfärgade lymfbanorna för att hitta portvaktskörtlarna. Fördelen med denna metod är att det kirurgiska ingreppet är betydligt skonsammare och att risken för kvarstående besvär från armen i form av känselnedsättning, rörelseinskränkning, smärta och armsvullnad minskar. Dessutom så undersöks portvaktskörtlarna noggrannare än övriga lymfkörtlar och därmed minskar risken för att missa tumörspridning till lymfkörtlarna. Idag är portvaktskörtelkirurgi standard för stadieindelning av tidigt upptäckt icke spridd bröstcancer. Det övergripande syftet med denna avhandling var att undersöka portvaktskörtelkirurgins roll vid bröstcancerbehandling idag med fokus på kvarvarande begränsningar i olika kliniska situationer samt om resultaten från de olika ingående delarbetena kan ha inverkan på dess framtida indikationer.

De specifka frågeställningarna var:

1. Hur vanligt är det med spridning till portvaktskörtlarna vid förstadium till bröstcancer (DCIS), som innebär att tumörcellerna växer på plats i mjölkgångarna men inte har börjat infiltrera sin omgivning?
2. I vilken omfattning ändras lymfavflödet från bröstet till armhålan efter en mindre diagnostisk bröstoperation? Går det att göra portvaktskörtelkirurgi i en andra seans?
3. Kan portvaktskörtelkirurgi även användas för stadieindelning av kvinnor med bröstcancer som skall få cellgifter/kemoterapi före sin bröstoperation (neoadjuvant) och kan denna stadieindelning göras innan start av neoadjuvant behandling (NAST)?

4. Kan portvaktskörtlekirurgi användas efter NAST för stadieindelning av kvinnor som hade konstaterad spridning till armhålan vid diagnos men där cellgifterna gjort att dessa metastaser i armhålan tillintetgjorts?

Det första delarbetet (paper I) är en registerstudie där alla kvinnor opererade för ett förstadium till bröstcancer i Sverige mellan 2008-2009 analyserades med avseende på utfört kirurgiskt ingrepp i armhålan och eventuella tumörceller i dessa lymfkörtlar. Av 1273 patienter hade portvaktskörtelkirurgi utförts i 753 fall varav fem hade metastas. Vi kunde inte påvisa några riskfaktorer för metastas i vårt material. Även eftersnittningar utfördes av tumörklossar från alla patienter med tumörceller i portvaktskörtlarna och ett dubbelt antal matchade kvinnor för att leta efter förbisedda millimetersmå områden där förstadiumet övergått till invasiv tumörväxt med förmåga att sprida sig i kroppen. Denna så kallade miktoinvasion påträffades i samma utsträckning i båda grupperna.

I delarbete två (paper II) undersöktes lymfavflödet till armhålans lymfkörtlar hos 37 kvinnor planerade för ett mindre ensidigt bröstingrepp för en förmodat godartad förändring utan behov av portvaktskörtelkirurgi. Veckan före, respektive sex veckor efter operationen, genomgick kvinnorna SPECT/CT- undersökning på Karolinska Universitetssjukhuset i Huddinge. Denna undersökning innebär att ett radioaktivt spårämne sprutas, i detta fall i båda brösten, och att bildtagning sker med start efter en timme med en kombinerad gammakamera och datortomograf som tillsammanns genererar tredimensionella scintigrafiska bilder med tydliga anatomiska riktmärken. Vi kunde inte se någon statistiskt säkerställd skillnad i överensstämmelse i lymfdränaget efter kirurgin på opererade jämfört med icke opererade sidor eller i andelen procedurer där en radioaktiv lymfkörtel kunde upptäckas på opererade jämfört med icke opererade sidor.

Delarbete tre och fyra (paper III och IV) baseras på en svensk multicenterstudie där tillförlitligheten och den kliniska nyttan med portvaktskörtelkirugi vid NAST utvärderades. På kliniskt lymkörtelfriska patienter utfördes portvaktskörtelkirurgi före start av NAST och lymfkörtelutrymning tillsammans med bröstkirurgi efter avslutad NAST, som nästan alltid utgjordes av cellgifter. 224 patienter analyserades i delarbete tre (paper III) och hos alla hittades en portvaktskörtel. Däremot var det nio kvinnor med frisk portvaktskörtel som trots detta hade sjuka lymfkörtlar i armhålan efter NAST, vilket ger en “falskt negativ kvot” på 7,4 %.
I delarbete fyra ingick 195 kvinnor som alla hade sjuka lymfkörtlar i armhålan innan NAST, konstaterat med finnålsbiopsi. Dessa kvinnor genomgick istället forsök till portvaktskörtelkirurgi efter avslutad NAST, tillsammans med bröstkirurgi och lymfkörtelutrymning. Hos 77,9 % påträffades minst en portvaktskörtel och i 14,1% var portvaktskörteln falskt negativ. Dock sjönk andelen falskt negativa till 4,0 % i de fall då två eller fler portvaktskörtlar hittades.

Sammanfattningsvis visar denna avhandling att kvinnor med förstadium till bröstcancer har en mycket låg risk för spridning till lymfkörtlarna och därför bör portvaktskörtelkirurgi enbart utföras i de fall man planerar att operar bort hela bröstet eller om det finns starka skäl att tro att tumören innehåller invasiva områden. Efter tidigare begränsad diagnostisk bröstkirurgi förefaller portvaktskörtelkirurgi tillförlitligt trots att lymfavflödet ändrar sig i viss utsträckning.Portvaktskörtelkirurgi utförd hos kvinnor före kemoterapi (NAST) är säkert. Hos kvinnor med konstaterad lymfkörtelspridning innan NAST bör axillutrymning utföras i de fall då endast en frisk portvaktskörtel hittas efter avslutad NAST.

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